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GERMINATIVE ENERGY OF LOTS OF CONIFEROUS-TREE SEED. AS RELATED TO INCUBATION TEMPERATURE AND TO DURATION OF INCUBATION[1]

FERDINAND W. HAASIS

(WITH FIFTEEN FIGURES)

Introduction

The rate at which heat passes into or out of the plant body varies continually and the temperature of the plant always tends to approach that of the surroundings, from which it usually differs but slightly. The temperature of the surroundings may consequently be considered as an index of the concurrent plant temperature, and environmental temperature (as of soil and air) is usually looked upon as one of the chief influences that determine physiological activity. It is becoming increasingly appreciated that the influences exerted by temperature upon organisms are themselves dependent, however, on concurrent influences of other kinds. Some of these non-temperature influences are primarily related to, or bound up in, the internal characteristics of the organism or tissue considered, while others are active more or less directly at the external periphery of the organism and consequently operate inward from the environment. Whether any individual seed may germinate at all, for example, or how rapidly the physiological processes of germination may occur, with a specified set of temperature conditions, clearly depends only partly on the temperature complex itself, for the kind of seed considered, its physiological condition, etc., must obviously be important among the controlling conditions. The germination response of any viable seed to a given temperature complex is dependent upon many environmental conditions other than those of temperature; with a temperature complex that would otherwise give rapid

[1] Botanical contribution from the Johns Hopkins University, no. 104.

germination, this process may be greatly retarded or even wholly inhibited if the rate of water supply or of water loss, the rate of oxygen supply, or the rate of supply (or of removal from the surrounding region) of poisonous or stimulating materials (such as salts and organic compounds) is either too high or too low for satisfactory development of the organism when under the influence of the particular temperature complex in question. This recently familiar line of thought leads inevitably to the proposition that no statement about the temperature relations of vital processes can be more than very superficially useful, in regard to the definite analysis of physiological control and causation, unless the concomitantly effective, non-temperature conditions as well as those of temperature, are adequately defined. Of course the definition or description of these background influences cannot as yet be very precise in many respects, but they need to be and they can be clearly enough set forth to make it possible for other experimenters to approach them to an adequate degree.

Any given environmental temperature complex has several characteristics. The intensity or degree of the temperature obtaining in or around the organism considered (conveniently expressed as degrees on a thermometer scale) is first thought of; we may say that a seed germinated at a temperature of 25° C. But the temperature of a germinating seed is seldom even approximately maintained for any considerable period of time and temperature fluctuation consequently requires special consideration. Temperature may fluctuate very little (being nearly maintained) or it may fluctuate greatly and the fluctuations, whether small or large, may be slow or rapid in regard to time, often differing in rate for different portions of the experiment period. As has been emphasized by MacDougal (**14**), if the temperature is not practically maintained the form of the thermographic curve or tracing for any time period may be fully as important physiologically as are the corresponding minimum and maximum temperature values. Finally, whatever temperature complex is dealt with, whether the temperature of the organism is nearly maintained or fluctuates according to some adequately described pattern, the duration factor is of course of prime importance. Any statement of a physiological temperature relation must be considered as holding only for the exposure period for which that statement is made. We may say that a certain seed germinated with a maintained temperature of 25° (with regular or irregular fluctuations of plus or minus 1°, for example), but our statement is without much value unless we add that germination occurred within a certain number of hours, days, etc., following the beginning of incubation. Some of these considerations of the nature of temperature influence on organisms have received attention from Fawcett (**6**).

Technique and rational analysis have not yet progressed sufficiently to encourage the undertaking of serious experimental studies on the influence exerted on physiological processes by different forms of temperature fluctuation, and most of the investigations thus far reported in physiological literature have dealt with approximately maintained temperatures. From the point of view of ecological science (including forestry, agriculture, hygiene, etc.), for which natural conditions must be studied, temperature change has received some attention, but before the influence of fluctuating temperature can be approached by analytically planned experimentation it will be necessary that our knowledge of the much simpler maintained-temperature relations of organisms and tissues be greatly increased.

Many aspects of plant temperature relations are of great importance in plant ecology and the applied sciences that rest on plant physiology. This is especially true in regard to seed germination, for the germinating seed is usually so intimately and simply related to its surroundings that its temperature relations offer excellent opportunities for experimental study and for the application of physiological principles. Also from the standpoint of physiology itself seed germination is of exceptional interest, for it presents fewer difficulties than most of the other phases of plant activity and the complexity of its relations is correspondingly less discouraging to the experimenter. Furthermore, seed germination forms the starting-point for many different kinds of experiments upon the conditions that influence other plant processes, and precise knowledge and exact appreciation of the causal controls of germination are required for the working out of standard treatments calculated to produce a number of physiologically similar plants for experimental study. Experimentation on any aspect of plant activity is not apt to advance either rapidly or far until it is possible to secure at will lots of plants that are nearly enough alike for the special requirements of the investigation in hand. It may be added that the germinating seed furnishes us with a very compact and relatively simple vital system, which embraces most of the essential physiological processes. Seed germination and the growth of young seedlings have always been a favorite field for experimentation of both the cruder and the more refined types.

Comparatively few studies have been made on the temperature relations of seed germination from the standpoint of the fundamental analysis of determining conditions or controls, and the work here reported was planned to throw a little additional light into this particular corner of plant physiology and to furnish some aids and sign-posts that may be more or less useful to future workers who may wish to explore this field more thoroughly. From the nature of the problems involved, this study is of course of only

preliminary and exploratory nature. The concrete or immediate object was the determination of the influence of maintained temperature, acting for various lengths of time, upon the germinative energy of lots of seed of several different species of coniferous trees, especially pitch pine (*Pinus rigida* Mill.), the percentage of germination occurring under the test conditions being considered as the index of germinative energy.* A fairly extensive study of rice seed (*Oryza sativa* L.) was also made and seed of other agricultural plants was studied to some extent, but only the results obtained with seed of certain pines and other conifers will be presented in this publication.

It was especially desired to find out the optimal temperature for the occurrence of a high percentage of germination under the general conditions of the experimental tests and with different lengths of the incubation period, but attention was also paid to minimal and maximal temperatures and to the forms or patterns of the temperature-germination graphs. An attempt was made to employ more or less standard treatments of the seed samples with regard to the many influential conditions aside from temperature and the duration of the incubation period, but the aim of the study was not to find out just how to specify an environmental complex that would produce high percentages of germination in various lots of seed. That would constitute a practical problem that might promise the possibility of more immediate application in forestry and agriculture, but it might prove to be too complex for attack until a number of much more restricted and scientifically fundamental questions had been already answered much more clearly than is now possible. Indeed, the present study may be considered as an attack on one, or a very few, of the great array of such fundamental questions that must occur repeatedly to every student of physiology and the related applied sciences. It is hoped that the non-temperature conditions of the environment that were effective in these experiments are clearly enough described, in terms of the technique by which they were secured, so that other experimenters may be able to approach them to a fairly satisfactory degree. For some maintained temperatures and for some lengths of incubation period, the technique used surely furnished very satisfactory environmental complexes for the germination of large proportions of the main lots of seed studied.

Several matters of definition and logical analysis deserve attention at the outset. Following the usage of seed testers, *germination percentage* or the *percentage of germination* denotes the average number of seeds that germinated per hundred seeds used in any test. A sample of seeds is ex-

* This use of the word *energy* may be confusing, since the word is not here employed in its physical sense, but "germinative energy" has been used in discussions of seed testing and is recommended by TOUMEY (22).

posed to the influence of an environment calculated to induce germination in some of them at least, the number found to have germinated at the end of a certain period of time is ascertained, and this number is expressed as a percentage of the number of seeds in the sample used. For any test, this numerical value is considered as an index of the germinative energy of the sample tested and, if the sample fairly represented the entire lot of seed from which it was taken, the germinative-energy index of the sample is taken as the germinative-energy index of the lot. Several samples of any lot of seed are generally subjected to like tests and the germinative-energy index of the lot is the average of the indices of the samples. On the basis of any particular kind of viability tests a given lot of seed is to be considered as made up of two categories of individuals, (A) those that are capable of germination and (B) those that are not. If, for example, ninety per cent. of the seeds in a lot are individually viable for the kind of test used we say that the viability of the lot is 90 per cent. on the basis of that kind of test, or that the percentage viability of the lot is 90, and that value is the index of the germinative energy of the lot for the particular environmental complex used in the tests from which it is derived.

The viability of a lot of seed thus exhibits a special kind of variability, with reference to the innate capacity of the lot to produce seedlings, and with reference to a specified set of environmental conditions. This concept is not to be confused with those of growth rate, respiration rate, etc., as such process rates are dealt with in physiological literature. The lot index of viability does not represent a rate, it represents merely the proportion of the seed population that are individually viable under the conditions of the test. Process rates, on the other hand, such as the rate of elongation of seedling roots, for instance, have not been generally studied with primary regard to the innate variability of the lots of seedlings or plants studied. Indeed, most experimenters with process rates subject their experimental material to somewhat careful selection before each series of tests is begun, the aim being to secure as high a degree of uniformity in seeds, seedlings, etc., as is possible. Thus, seeds are often selected for uniformity in size, color, weight and specific gravity, and seedlings are frequently selected for size, form, color, for previous rate of growth or other physiological capacity, etc. Such preliminary selection is essential in studies on the environmental control of physiological processes, as every one knows, for the results of such studies are valid only for the particular internal conditional complex that was represented by the lot of plants for which the results were derived. Because satisfactory uniformity in any lot of material is difficult to secure, it remains desirable, however, to give special attention to variability, even after uniformity has been approached as nearly as possible by various kinds

of preliminary selection. It is this thought that has led to the employment of elaborate statistical methods for the examination of experimental results that lend themselves to such treatment. Innate variability implies internal differences in physiological capacity, and hence in performance or behavior, among the several individuals of an experiment, and it constitutes at present perhaps the most serious obstacle to physiological and ecological progress. This statement applies especially to the logical analysis that is so important in the planning of experimental procedure as well as in the interpretation of experimental results.

Since innate variability is to be reduced as much as possible in experimentation on physiological control and since it is always present, in spite of everything an investigator may do, it becomes itself an important and very fundamental characteristic of any group of organisms, such as seeds or seedlings, cuttings or scions, etc. It requires attention and measurement at every turn. But variability is not a characteristic of the individual unit in an experimental series; it is of course a characteristic of the group only. In the sense here considered a single seed, for example, does not manifest variability. An individual seed possesses size, color, a certain amount of capacity for germination under any set of environmental conditions, etc., and the innate variability of a sample of seeds, however it may be measured, depends on the manner in which the several individuals of the sample differ from one another; an index of variability must characterize the physiological differences that obtain in the group. Knowledge of this group characteristic should permit selection for uniformity with regard to the several kinds of variability and should facilitate the interpretation of the numerical results of experiments on physiological processes. As has just been implied, such knowledge is also desirable whenever different lots of individuals are to be compared, as in ordinary seed testing, for example. The aim of the seed tester is not primarily to ascertain just how individual seeds differ, but how one lot of seed differs from another lot with respect to capacity to produce seedlings. Individual differences are studied primarily to obtain information by which the lot differences may be compared.

An index of germinative energy needs always to be stated with specific reference to the particular complex of environmental conditions with which the germinative-energy tests were made, for the index value cannot be considered as valid except for that complex. It is clear that the numbers of seeds germinating in two like samples and the germination percentages derived therefrom may be very different if the two samples are tested in different ways. Water conditions, oxygen conditions, temperature conditions, etc., are all influential, and especially the duration of the test period. For any lot of seed, for any set of non-temperature conditions and for a

stated length of the test period, the index of germinative-energy may have widely different values according to the temperature conditions of the test; for any lot of seed and for a stated set of physical and chemical conditions in the test, the value of the index of germinative energy may differ greatly according to the length of the period of incubation, etc. In short, the magnitude of the germinative-energy index is a function of a large number of variables, of which only a portion represent characteristics of the lot of seed considered, while the rest stand for the environmental conditions of the test and its duration.

Because of these considerations the germination percentage derived from any test of a sample of seeds is to be regarded only as corresponding definitely to the conditions and duration of the test. The index value may have any magnitude between zero and a maximum, the upper limit being determined by the characteristics of the lot of seed in question. It is consequently essential that we try to understand what are the environmental influences that affect the index value for any lot of seed and just how they operate to give that value any particular magnitude. Such an investigation as is here suggested may be approached only by means of analytically planned experimentation. For the present, most of the experimental conditions have to be relegated to the category of background conditions (to be described as adequately as possible and made uniform throughout the several tests of an experiment) while a very few may be considered, in any case, as experimental variables (to be studied with regard to differences in their intensities and qualities and always with special reference to the chosen background complex).

In the experiments dealt with in this paper all influences were in the background excepting (1) lot of seed, (2) maintained temperature and (3) duration of incubation. It will be seen in this report, for example, that the first lot of pitch pine seed tested gave, for the standard set of background conditions employed, many different indices of germinative energy, ranging from zero to about 90, the index values being environmentally influenced (always with specific reference to this particular set of background conditions) by temperature and duration of incubation. For shorter periods of incubation (not too short) the values are all low but they are higher with some maintained temperatures than with others. For longer periods of incubation they are very high with some maintained temperatures and very low with others. Whether the highest percentage of germination shown approaches the physiological and statistical limit for that lot of seeds remains questionable, but (since the highest value is close to 100 per cent.) it is clear that the maximum viability percentage was nearly attained.

An interesting feature of these results is the manner in which the form of the temperature-germination graph changes with the length of the incubation period, and it appears that the innate nature of any lot of seeds cannot be well understood unless this feature is experimentally studied. The optimal maintained temperature for the occurrence of germination in a given sample of seeds (or the temperature giving the highest index of germinative energy for that sample and for the test conditions) is not at all the same for short incubation periods as for longer ones, unless all periods are relatively long.

The individual seeds of any lot differ as to germinating power, and experimentation of the sort employed in these studies furnishes data for classifying the individual seeds in this respect. The results show what percentage of the lot (how many per hundred, on the average) may be expected to germinate under the influence of each set of test conditions (the duration factor being considered as one of the test conditions) and also what percentage of the lot may be expected to fail to germinate under the influence of each set. Failure to germinate does not necessarily imply lack of viability in a seed. Seeds that fail to germinate under one set of conditions may of course germinate under another set. For any given set of test conditions and for any incubation period, the seeds of any sample may thus be classified into germinable and non-germinable. For any set of non-temperature conditions and for any incubation period they may be classified further into several categories with regard to temperature. This question of the experimental classification of a lot of seeds is referred to in a subsequent section.

Different lots of seed are of course to be expected to differ in regard to statistical make-up, in spite of their being of the same species or variety, from the same individual plant, from the same inflorescence or even from the same capsule. Such differences between lots of seed, which are the differences with which a seed tester deals, are partly due to differences in the original proportions of "good" and "bad" seeds when the lots were harvested. Also, since stored seed is apt to deteriorate with time, the germinative-energy index of a given lot (for a particular kind of test) may alter more or less rapidly with time, according to the original make-up of the lot and the conditions of storage.

The analytical study of the temperature relations of vital phenomena has been approached by various methods and this whole field of science was reviewed, up to 1897 and 1914, respectively, by DAVENPORT (**3**) and KANITZ (**8**). A number of studies on the temperature relations of plants have dealt with the rate of elongation of roots and shoots of seedlings after germination has occurred. Examples are afforded by the studies of SACHS

(19), KOEPPEN (9), DE VRIES (4), LEHENBAUER (11), ISABELLA LEITCH (12) and NEWCOMBE (15). Since the studies considered in the present paper do not deal directly with rates of growth, they are not closely related to the contributions here cited and it is not necessary to present in this place a résumé of the latter.

Some investigators have studied the germinative energy of lots of seed as indicated by the percentage of germination when samples of each lot were exposed to the same or different environmental complexes for various incubation periods. Studies of this kind are somewhat more closely related to the present studies than are those dealing with rates of shoot or root enlargement, but the earlier results offer but little opportunity for analytical comparisons with those set forth in the present publication. Productive comparisons are generally precluded by the nature of the plans of experimentation that were followed. In this group may be mentioned reports by NOBBE (16), RAFN (18), DOROSHENKO (5), PACK (17), HARRINGTON (7), SIFTON (21), and especially those by BOERKER (2) and KORSTIAN (10).

NOBBE (16) aimed to find out what are the most suitable temperature conditions for germinative-energy tests of certain forest-tree seeds. He employed maintained temperatures and also studied the influence of alternations of maintained temperature. In the latter case a sample of seeds was exposed to the action of two different maintained temperatures, for different parts of the incubation period. His background conditions (considered as uniform for all cultures) were those of moisture and oxygen supply and his variables were temperature, length of incubation period and kind of seed. These studies were of course planned for practical application in seed testing rather than for the analysis of physiological control. The studies of HARRINGTON (7) were similar to those of NOBBE.

RAFN (18) aimed to have as background conditions all influences excepting kind of seed and duration of the incubation period. His studies included lots of seed of more than two hundred different tree species. DOROSHENKO (5), PACK (17) and SIFTON (21) employed as variables maintained temperature, duration of the incubation period and kind of seed.

BOERKER (2) studied the germinative energy of a number of kinds of tree seeds and considered as variables the kind of seed (species, race, size, etc.), the duration of the incubation period, the intensity of illumination (natural light modified by shade, in a greenhouse), soil-moisture content and soil texture. He gave little attention to temperature, however, and his air-temperatures, above the seed bed, fluctuated in irregular manner, between about 8° and about 38° C., when measured in the shade. The extent of the weekly temperature range was never less than 16° and it was

sometimes as great as 29°. His experiments lasted about six months and he regarded the temperature conditions as optimal throughout that period. The results are presented as graphs, in which ordinates represent percentage of germination and abscissas represent the length of the incubation period expressed in days.

KORSTIAN (**10**) studied the germinative energy of acorns of several species. His variables were the kind of seed (species), the duration of the incubation period (measured in five-day units) and the temperature conditions, other influences being about uniform for all tests and having values not far from what he regarded as optimal for the seeds studied. His temperature conditions were generally not those of maintained temperatures, though some tests were carried out in a cold-storage room at about 4°. For most of the tests he employed a definite type of fluctuating temperature involving what may be called alternating maintained temperatures; the cultures were transferred twice daily (morning and evening) from one incubator to another, two incubators (each with its own maintained temperature) being employed for any given test. The pairs of maintained temperatures employed were 2° and 10°, 10° and 18°, 18° and 27°, and 27° and 35°. Thus, for example, a culture was subjected to a maintained temperature of 18° during the day time, the temperature was then quickly changed to 10° and the culture was subjected to this lower maintained temperature overnight, the temperature being again quickly changed back to 18° on the following morning, etc. KORSTIAN's results are presented in graphs constructed similarly to those of BOERKER.

Although the present study was conducted along lines somewhat like those followed in KORSTIAN's work, the two plans were essentially different in many fundamental respects, as will appear later. Only maintained temperatures were employed in the present work, excepting as temperature fluctuation was necessitated by the short observation periods (when the cultures were outside of the incubators) and the background conditions were arranged so as to be reproducible with unusually close approximation. The problem, as set up in planning these studies, is much less complex than has generally been the case in earlier studies on the germinative energy of lots of seed and the environmental complexes for which the results are to be considered as valid are here perhaps more closely defined than has thus far been customary in this kind of experimentation.

The main features of this study of conifer seeds may be summarized as follows: The general or background conditions were considered with some care and a sort of standard technique was worked out for them. Light was excluded from the cultures excepting as they had to be removed from the culture chambers for observation. Many different maintained tempera-

tures were employed and many different lengths of the time period of incubation for each maintained temperature. To ascertain the percentages of germination for different environmental complexes and for different initial physiological or internal complexes (lots and kinds of seed) was the immediate aim of the experimentation. The work was planned primarily to advance our knowledge of principles or generalizations that may express the causal relations of maintained temperature and its duration to the percentage of germination occurring in the lots of seed tested; that is, to the germinative energy of each lot as manifested under the conditions of the several tests.

The study here reported was carried out betewen October, 1927, and March, 1928, in the Laboratory of Plant Physiology of the Johns Hopkins University, where the author occupied a position as student assistant. The work was made possible by a furlough granted the writer by the United States Department of Agriculture, which allowed him to be absent from his post with the Appalachian Forest Experiment Station during the academic years of 1926–27 and 1927–28. For himself as well as for the Johns Hopkins Laboratory of Plant Physiology, he wishes to mention with appreciative thanks the kindness of those who supplied the seeds used in the work. Their names are recorded on a subsequent page. For useful information about molds that were encountered in the cultures cordial thanks are expressed to Doctor CARL HARTLEY, who was once a student in this laboratory, to Mr. L. W. R. JACKSON, both of the United States Bureau of Plant Industry, and to Doctor CHARLES THOM, of the United States Bureau of Chemistry and Soils. The author wishes to express his thanks to Doctor W. H. TISDALE, of the Jackson Laboratory of the E. I. du Pont de Nemours Company, for supplying samples of the antiseptic "Semesan" and communicating valuable information about its composition and use. Finally, he desires to acknowledge his indebtedness to Doctor BURTON E. LIVINGSTON, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for constant aid and criticism in the planning and conduct of the experiments, in the logical analysis of the results and in their presentation. He is also greatly indebted to his wife, BESSIE AMERMAN HAASIS, for helpful suggestion and criticism throughout the course of the work.

Experimental methods and materials

STANDARD CULTURES AND THEIR TREATMENT

The cultures of this study may be conveniently grouped in two categories, (1) the standard cultures and (2) a number of preliminary or supplemental additional cultures of other types. The standard cultures were all prepared and treated in the same general manner and they and their

treatment will be described first, after which the additional cultures and their treatment will be briefly described, largely as modifications of the standard type.

The seeds were germinated on agar plates in covered glass Petri dishes 9 cm. in diameter and 1 cm. deep, which had been held at a temperature of 140°–160° C. for at least an hour in an electric oven. Sufficient granulated agar ("Bacto-Agar," from the Digestive Ferments Co., Detroit, Michigan) for the entire investigation was thoroughly mixed before work was begun, to form a single stock of this material. Very close similarity in the many lots of agar solution that were to be prepared during the course of the experimentation was thus insured. A stock of "Semesan" (from the E. I. du Pont de Nemours Co., Wilmington, Delaware, described as containing 35 per cent., by weight, of hydroxy-mercuri-chloro-phenol, the balance being about one-third $CaCO_3$ and two-thirds Na_2CO_3) was prepared by mixing in the same way. The water used was from a "Barnstead" still.

The agar plates were prepared as in bacteriological procedure. The granulated agar was poured into the water while the latter was boiling and the mixture was stirred till all agar particles had disappeared. A gram of dry agar was used for each hundred cubic centimeters of water. Different lots of this mixture were made from time to time, for the several series of plates. Twenty cubic centimeters of the liquid agar mixture was used for each standard plate, being pipetted into the Petri dish while hot. Each dish was then immediately covered and allowed to cool.

The seeds, which had been previously soaked in antiseptic solution, were distributed uniformly on the surface of the agar plate, one hundred seeds to a plate. The seeds were arranged in a square pattern of ten rows, ten in a row, at distances of 6 mm. each way. Uniform spacing, secured by using a guide diagram or pattern on paper lying under the dish while the seeds were being distributed, obviated the necessity for tedious counting. The soaked seeds were placed by means of forceps, which were rinsed in standard "Semesan" solution from time to time while the work of distributing the seeds was in progress. The seeds quickly adhered to the agar gel sufficiently to allow the cultures to be freely handled and soon inverted without alteration in the arrangement pattern.

Because preliminary tests indicated that the seeds in an experiment were apt to become moldy before germination occurred, they were regularly soaked, for an hour just before being distributed on the plates, in a solution of "Semesan." This preliminary treatment was found to retard the development of mold. It should be noted that the antiseptic treatment introduces special considerations and may complicate to some extent the analysis of the experimental conditions. The treatment may possibly have resulted

in some cultures giving a somewhat lower percentage of germination than they would have given if the seeds had been soaked in distilled water, but the evidence at hand indicates that such chemical influence was never significant. Without the antiseptic treatment the influence of mold would have been very serious with some of the sets of test conditions.

The standard "Semesan" solution used for preliminary soaking was prepared with one gram of the powder for each 400 cc. of water. Since the proprietary product is stated to contain 35 per cent. by weight, of hydroxy-mercuri-chloro-phenol, this standard solution is taken to contain 0.0875 gm. of this compound in each hundred cubic centimeters. Fresh lots of solution were prepared at intervals as required and the solution was never used when more than five days old.

Most of the seeds tested failed to sink in the antiseptic solution and a special technique was consequently required for the preliminary soaking. Each batch of seeds was soaked in a large-mouth, cork-stoppered bottle, of 250 cc. capacity, nearly filled with solution so as to contain only about 1 cc. of air. During the soaking period the bottles were continuously rotated about a horizontal axis (three or four turns a minute), being so fixed that the long axis of each bottle remained inclined about ten degrees from the axis of rotation. By the rotating of the bottle and the quick movement back and forth of the air bubble adjacent to the seeds, these were kept in constant motion during the period of soaking. Each seed was thus continually bathed in solution and the adjacent solution was continually renewed.

About a thousand seeds were soaked for each series of cultures. At the end of the soaking period the wet seeds were spread out on the plane bottom of a glass dish and were then transferred, one by one, to the agar plates. Only apparently healthy seeds were used, but no attempt was made to select them for size, color, etc., the chief aim being to obtain similar groups of one hundred seeds each, so that all cultures of the same lot of seed might be comparable. Seven cultures were usually included in each series, one for each of the seven different maintained temperatures that were available.

The time required for the distribution of the seeds in a series of cultures was generally less than an hour, being shorter as experience and practice increased. Toward the end of the study the seeds for a series of seven cultures were generally placed on the plates in half an hour. Soaking and placing both occurred at room temperature, generally between about 19° and about 22° C. On account of evaporation, the seeds were probably a little cooler than the air when placed on the plates. It is thus seen that each experiment started by soaking all seeds in standard "Semesan" solution for from 1.5 to 2 hours, with temperatures around 20°. As soon as

the preparation of a series of cultures was completed the cultures were distributed in the maintained-temperature chambers, so that the differences between the temperatures began to be effective about 1.5–2 hours after the seeds were put to soak. Since germination of the seeds studied is relatively slow at temperatures around 20°, it may be supposed that the pregermination processes had not progressed far during the time period devoted to soaking and distribution. The general consistency of the results obtained indicates that the latter would not have been notably different if the preliminary period had been shorter or if it had been characterized by lower temperatures or by smaller temperature fluctuations.

The maintained temperatures

The battery of controlled-temperature chambers used in this study was described in its main essentials by Livingston and Fawcett (**13**) and its operation was referred to by Fawcett (**6**), for whose studies on the temperature relations of some parasitic fungi it was originally constructed. A number of investigations have been carried out with the aid of this series of chambers in the last twelve years. The apparatus was overhauled in the fall of 1926 and mechanical refrigeration was introduced at that time, to replace ice cooling, which had been used before. The ice-water tank is now filled with calcium chloride brine (about equal parts by weight of the commercial salt and water) and this solution is cooled by a mechanical refrigeration unit such as is commonly employed in home refrigerators. The form used has an electric motor that intermittently operates a piston compressor, sulphur dioxide is the refrigerant and the apparatus is cooled by flowing water.

The introduction of mechanical refrigeration has rendered more convenient the employment of temperatures below that obtained with melting ice, but the mechanical unit used has not been without occasional lapses and need for expert attention and it has perhaps not been, on the whole, more satisfactory than was the daily addition of ice, especially when it has been required that the temperature of the cooling tank should approach room temperature. The features requiring attention (not always at convenient times in an experiment series) have been the motor itself and the thermostatic switch. Only once, at the time of installation, has any notable escape of sulphur dioxide occurred and that accident might have done no harm had the apparatus been located elsewhere than in a greenhouse full of living plants, for of course no cultures were in the chambers at the time. It is suggested that a refrigeration apparatus of the absorption type, without motor or pump, may possibly prove in many cases to be more suitable for use in scientific experimentation than those forms that depend on

mechanical devices for compressing the refrigerant. The series of chambers is located in one of the greenhouse rooms of the laboratory because of the lack of other more suitable space and the air temperature of the room fluctuates considerably. A basement room, preferably with automatic temperature control to within a few degrees of fluctuation, would be preferable to the present location.

The seven culture chambers of this series are cylindrical (38 cm. in diameter and 38 cm. deep) and each opens above. They are arranged in a row, each being provided with its own water-jacket, which has a motor-driven stirrer. Supported from the top of each chamber is another cylinder, of thin, multi-perforated galvanized sheet iron, 28 cm. in diameter and 43 cm. high, with its floor about 2 cm. above the floor of the outer cylinder. The 5-cm. layer of air between the outer and inner cylinders is kept in motion by two vertical stirrer rods, each of which is bent upon itself to form an inverted U, the terminal portion of which operates to stir the water of the jacket. The water-jackets of adjacent chambers are separated by sheet-iron partitions, which prevent any movement of water from jacket to jacket but allow heat transfer by conduction. At each end of the series is a control tank with stirrers and each of these tanks is separated from the jacket of the adjacent chamber by a sheet-iron partition. One of the tanks (at the hot end of the series) contains water, heated by means of an electric heater with thermostatic control. The control tank at the other (cold) end of the series, which also has stirrers, contains calcium-chloride brine and has thus far been operated without heating apparatus. The sheet-iron partition between it and the cooling tank (where refrigeration is applied) has perforations which, in these studies, have been only partially closed by rubber stoppers, so that circulation of brine might occur between these two tanks. The thermostatic regulation of the temperature of the control tank at the cold end has thus been left to the refrigeration apparatus. The water of the chamber jackets and that of the hot control tank is covered by a thin layer of engine oil, to retard evaporation. Sides, bottom and ends of the series as a whole are insulated with a layer of tightly packed oxhair (such as is used in plaster for the interior of buidings) and the top, including the removable covers of the several chambers, is insulated by pine wood and about 3 cm. of sheet cork. The whole is covered above, in addition, by pads of saddler's hair-felt 3 cm. thick, two thicknesses of ordinary wool bed-blankets and a white canvas tarpaulin. In spite of this extra insulation for the tops of the chambers, it appears that most of the heat leakage occurs through this region. A considerable amount of heat leakage is of course inevitably due to the opening of the chambers for observations. Visible radiation is completely excluded from the chambers, and the cultures were therefore in darkness excepting when observation was necessary.

For any set of seven maintained temperatures (or of narrow temperature ranges, to be more precise) it is necessary only to adjust the two thermostats that control the supply and the removal of heat at the ends of the series. A day or two of idle operation follows each new setting, to allow the chambers to approach dynamic equilibrium with one another and with the control tanks, after which each one maintains its temperature indefinitely, generally with a fluctuation of less than a single centigrade degree above and below the mean. The temperature fluctuations of the cultures were of course greater in most instances, on account of their being removed at intervals for observation, but the periods of departure from the mean on this account were kept as short as possible.

The difference between the temperature of the water or brine in either control tank and the air temperature in the adjacent chamber was about 10°, being somewhat larger or smaller according to the settings of the thermostatic controls. The difference between the air temperatures of any two adjacent chambers was of course determined by the two control temperatures in any setting. It was not uniform throughout the series, being usually smaller toward the colder end.

Each chamber is provided with a calibrated thermometer of the ordinary chemical type, which may be momentarily raised and read without disturbing the chamber cover. Temperatures were observed and recorded at frequent intervals in each period of operation and small Richard thermographs were operated in some of the chambers, while maximum-minimum registering thermometers of the Six type were used in some cases. From the available temperature records a mean was derived as accurately as possible for each chamber for each period of operation, to represent the maintained temperature of that chamber for the period in question. The means for eleven periods of operation, considered as the respective maintained temperatures dealt with, are shown in table I, where the eleven periods are numbered chronologically and the seven chambers are numbered serially, beginning at the cold end of the series.

It will be seen from the data of table I that the same chamber was used, at different times, for different maintained temperatures, and that the same maintained temperature was in many instances obtained in different chambers at different times. No relation was discovered between the seed responses and the particular positions (in the greenhouse) of the chambers in which they occurred, and the use of different chambers for the same maintained temperature need not be considered further. Similarly, no relation was observed between the seed responses and the periods of operation in which they occurred; hence the temperature means for all periods of operation of the control chambers may be considered in a single series,

TABLE I

PERIODS OF OPERATION OF MAINTAINED-TEMPERATURE CHAMBERS AND MEAN TEMPERATURES OF THE SEVEN CHAMBERS FOR EACH PERIOD

PERIOD OF OPERATION		TEMPERATURE MEANS						
PERIOD NO.	DATES (1927–28)	1ST CHAMBER	2ND CHAMBER	3RD CHAMBER	4TH CHAMBER	5TH CHAMBER	6TH CHAMBER	7TH CHAMBER
		Deg. C.	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*
1	Oct. 14–26	20	25	31	35	42	48	55
2	Oct. 31–Nov. 16	16	20	24	27	33	37	41
3	Nov. 16–18	18	21	26	29	34	37	42
4	Nov. 25–Dec. 8	26	29	33	36	41	45	51
5	Dec. 16–24	8	12	15	18	22	24	28
6	Dec. 29–Jan. 8	20	25	31	36	43	50	57
7	Jan. 11–24	20	24	28	32	36	41	46
8	Jan. 28–Feb. 4	23	26	30	35	39	44	50
9	Jan. 30–Feb. 11	26	28	31	36	40	45	50
10	Feb. 25–Mar. 9	18	22	26	30	36	39	45
11	Mar. 3–27	18	22	26	30	36	40	46

as though all tests had been made simultaneously. The following list, compiled from table I, shows the maintained temperatures actually employed, together with notations as to the corresponding periods and chambers. The period numbers, with subscripts to indicate chamber numbers, are shown in parentheses for each temperature.

8° (5_1)	33° (2_5, 4_5)
12° (5_2)	34° (3_5)
15° (5_3)	35° (1_4, 8_4)
16° (2_1)	36° (4_4, 6_4, 7_5, 9_4, 10_5, 11_5)
18° (3_1, 5_4, 10_1, 11_1)	37° (2_6, 3_6)
20° (1_1, 2_2, 6_1, 7_1)	39° (8_5, 10_6)
21° (3_2)	40° (9_5, 11_6)
22° (5_5, 10_2, 11_2)	41° (2_7, 4_5, 7_6)
23° (8_1)	42° (1_5, 3_7)
24° (2_3, 5_6, 7_2)	43° (6_5)
25° (1_2, 6_2)	44° (8_6)
26° (4_1, 3_3, 8_2, 9_1, 10_3, 11_3)	45° (4_6, 9_6, 10_7)
27° (2_4)	46° (7_7, 11_7)
28° (5_7, 7_3, 9_2)	48° (1_6)
29° (3_4, 4_2)	50° (6_6, 8_7, 9_7)
30° (8_3, 10_4, 11_4)	51° (4_7)
31° (1_3, 6_3, 9_3)	55° (1_7)
32° (7_4)	57° (6_7)

No special attempt was made to control the air composition in the culture chambers nor in the culture dishes themselves. The air over the agar plate must have been generally nearly saturated with water vapor and the germination data indicate clearly that the supply of oxygen (or the disposal of carbon dioxide) was in no case a limiting condition. It should be noted that the covers of the culture chambers and those of the dishes were surely not tight enough to seriously retard gas diffusion and that the air of the chambers generally approached water saturation.

Special cultures and their treatment

Besides the standard cultures, which were used in most of the tests, a number of preliminary and supplementary tests with modifications of the standard culture methods were employed. In some of these less than 100 seeds were used for each culture. In some instances dry seeds were placed on the plates without preliminary soaking, or they were soaked in tap-water or in distilled water instead of "Semesan" solution, or the concentration of "Semesan" and the period of soaking were different from that noted above, or some other antiseptic was employed.

For these special cultures the temperature chambers of the series described above were employed in some instances, and electric incubators were

used in other instances. The latter maintained mean temperatures of 25°, 30°, and 35°, with fluctuation of about one degree above and below the mean. A few cultures were transferred from the regular temperature chambers to incubators before observations were completed. Further details concerning these additional cultures will be presented farther on, as required in the presentation of the results.

Observations, records, computations and graphs

Cultures were usually placed in the chambers in the forenoon, within an hour of the placing of the soaked seeds upon the agar plates, and observations were usually made 21 hours later and at 24-hour intervals thereafter. No effort was made to keep the observation intervals exactly uniform for all cultures of the same series and there was some variation in their length. Cultures were usually prepared and placed in the chambers on several successive days after the beginning of each period of operation. The several series of any period consequently overlapped in regard to time.

Observations were made on small groups of cultures (not more than six), and the rest remained in their chambers till work with the group in hand had been completed and they had been returned. The chambers remained closed excepting momentarily, when cultures were removed or replaced. Observations were made in the shade in the greenhouse, the work being performed as rapidly as possible. All cultures were consequently exposed to conditions other than those of the culture chambers for a short time whenever observations were made. As much as an hour and three quarters might elapse between the observation of cultures from the first chamber and the observation of cultures from the seventh chamber. Observation regularly began with the coldest chamber and proceeded along the series of chambers to the hottest one.

Observation intervals were not always the same for different series. Intervals of a few hours were used in a number of experiments. In a few cases different series were started at 2-hour intervals, so that observations made at about the same time might give data for intervals of several different lengths; *e.g.*, for 10, 12, and 14 hours from the beginning of the test. For experiments in which the observations of the first day or two were to occur at short intervals, the number of cultures was so restricted that the examination of all of them might be completed in fifteen minutes. It is obvious that the number, frequency and distribution of the observations made on a given culture in an experiment period must have largely determined the number and distribution of the pronounced temperature and other environmental fluctuations to which that culture was inevitably subjected on account of its temporary removal from the chamber for observation.

The Petri dish remained uncovered for a few minutes during each examination of each culture and all germinated seeds were removed with forceps and counted at each examination, the points of the forceps being frequently dipped in standard antiseptic solution. A satisfactory background was afforded by a piece of black paper placed beneath the culture dish during the examination.

The attainment of germination was considered as having occurred as soon as a definite and clearly discernible projection of the embryo had pushed out from the ruptured seed coat. For the conifer seeds here considered this implies the protrusion of the primary root. This is an easily observed stage or phase of the germination process, arbitrarily chosen for convenience. A similar stage was employed by KORSTIAN (**10**) in his study of germinating acorns. If it seems desirable the attainment of this protrusion stage of germination may be considered as marking with a considerable degree of precision the transition from germinating seed to seedling, but there is not as yet any generally-followed convention in the use of these vague terms. Perhaps many students of seed germination would consider that germination begins with the onset of swelling and that the process continues till leaves appear. However that may be, in the present account the word germination implies simply the attainment of the protrusion stage.

Since observation of any culture could not be continuous and since undesirable environmental fluctuations became more pronounced with more frequent observations, many seeds had progressed far beyond the critical protrusion stage before germination was noted. At the end of a 24-hour period seeds of pitch pine, for example, might exhibit primary roots as much as 5 mm. in length. The inaccuracies thus introduced always tend to render the recorded incubation period for a seed, or the recorded mean period for a number of seeds, too long; it is never too short. Account was taken of these sources of inaccuracy wherever possible. They generally vanish or become insignificant for our present purposes, through the averaging processes to which all numerical data were subjected.

The number of seeds that had germinated in each culture since the last observation was ascertained and suitably recorded for each observation, and the total number that had germinated since the beginning of the test was then obtained for each incubation period, by summation. For example, a certain standard culture incubated at a mean temperature of 32°, which had shown no seeds germinated at the end of the first day, showed 10 germinated at the end of the second day, 30 more at the end of the third day, and 19 more at the end of the fourth day. The percentages of germination for the four different incubation periods were therefore as follows:

Length of incubation period	1 day	2 days	3 days	4 days
Percentage of germination	0	10	40	59

Each standard series thus gave a set of seven values or indices of germinative energy for each incubation period used, one value for each maintained temperature. These indices were plotted to give a temperature-germination graph, with abscissas representing maintained temperatures and ordinates representing the percentage values, and each length of incubation period naturally had its own graph. From a system of such graphs it was easy to observe what was the temperature giving the highest percentage for each length of incubation period, what was the general form of each graph and how the graph form altered from one length of period to another.

When the experimentation had been discontinued all comparable data for each lot of seeds were assembled in a single tabulation and all indices of germinative energy were averaged for each of the several tested maintained temperatures and for each length of incubation period. For example, there were ten values for lot 1 of pitch pine seed for the 3-day incubation period and for the maintained temperature of 41°. These were obtained from three periods of operation of the temperature chambers, as follows: (1) three values from as many cultures in the fifth chamber, (2) two values from two cultures in the sixth chamber, and (3) five values from five cultures in the seventh chamber. The ten comparable values (which ranged in magnitude from 12 to 34) were averaged to give the final average index for this lot of seed for the 3-day incubation period and for the maintained temperature of 41°. The final average index is 20 in this case. An average index was obtained in this manner for each of the remaining temperatures that had been employed for the 3-day period and these were plotted to form a graph for that length of period and for this particular lot of seed. Other lengths of incubation period for the same lot of seed were treated in the same general way, each length of period having its own graph, and these graphs were assembled on one sheet, with the abscissas the same for all.

Seeds used

The lots of seed with which this paper is concerned are listed below.

Pitch pine (*Pinus rigida* Mill.):—

Lot 1. Crop of 1926, New Jersey. (Received in June, 1927.[a])

Lot 2. Crop of 1926, New Jersey. (Received in December, 1927.[a])

Lot 3. Crop of 1927, New England. (Received in January, 1928.[a])

Lot 4. Crop of 1927, New Jersey. (Received in January, 1928.[a])

Lot 5. Crop of 1927, Franklin Co., Pennsylvania. (Received in February, 1928.[b])

Red pine (*P. resinosa* Ait.). Crop of 1927. (Received in December, 1927.[a])

Scotch pine (*P. sylvestris* L.). Crop of 1927. (Received in January, 1928.[a])

Lodgepole pine (*P. murrayana* Balf.). Crop of 1927. (Received in January, 1928.[a])

Long leaf pine (*P. palustris* Mill.). Crop of 1927. (Received in November, 1927.[a])

Western yellow pine (*P. ponderosa* Laws.). Crop of 1927, Hat Creek Forest, British Columbia. (Received in February, 1928.[b])

Loblolly pine (*P. taeda* L.). Crop of 1927, Pender Co., North Carolina. (Received in February, 1928.[b])

White spruce (*Picea canadensis* BSP). Crop of 1926. New England. (Received in June, 1927.[a])

Engelmann spruce (*P. engelmanni* Engelm.). Crop of 1926, Mt. Ida, British Columbia. (Received in January, 1928.[b])

Smooth cypress (*Cupressus glabra* Sudw.). Crop of 1927. (Received in November, 1927.[a])

Results and discussion

Pitch pine seed, lot 1, standard cultures

Data from standard cultures of lot 1 of pitch pine seed are assembled in table II, where each line is devoted to a single maintained temperature and each column is restricted to a single length of the incubation period. The number of cultures on which each mean germination percentage is based is shown as a subscript numeral following the mean value. These data are shown also by the nine narrow-line graphs of figure 1, there being a graph for each length of incubation period shown in the table. On these graphs abscissas represent temperature means, and ordinates represent percentages of germination. All the graphs are plotted on the same scale but with different base lines, the positions of the several different base lines being shown at the left of the figure ($0_{6-7\ hr.}$ to $0_{14\ da.}$). The temperature scale is shown at the bottom.

Examination of the graphs of figure 1 will make apparent the main contributions of the present paper. While the data on which the graphs are based are not as numerous and extensive as might be wished, yet they are generally consistent and their indications seem to be clear.

The maximal temperature was not reached; even incubation periods as short as 6 or 7 hours showed some seeds to have germinated with the highest

[a] Purchased from C. B. Fleu, Jr., Philadelphia.

[b] Lots marked b were kindly furnished as follows: Pitch pine, lot 5, by Mr. J. S. Illick, Harrisburg, Pa. Western yellow pine, by Mr. A. C. Thrupp, Kamloops, B. C. Loblolly pine, by Dr. C. F. Korstian, U. S. Forest Service. Engelmann spruce, by Mr. P. M. Barr, Victoria, B. C.

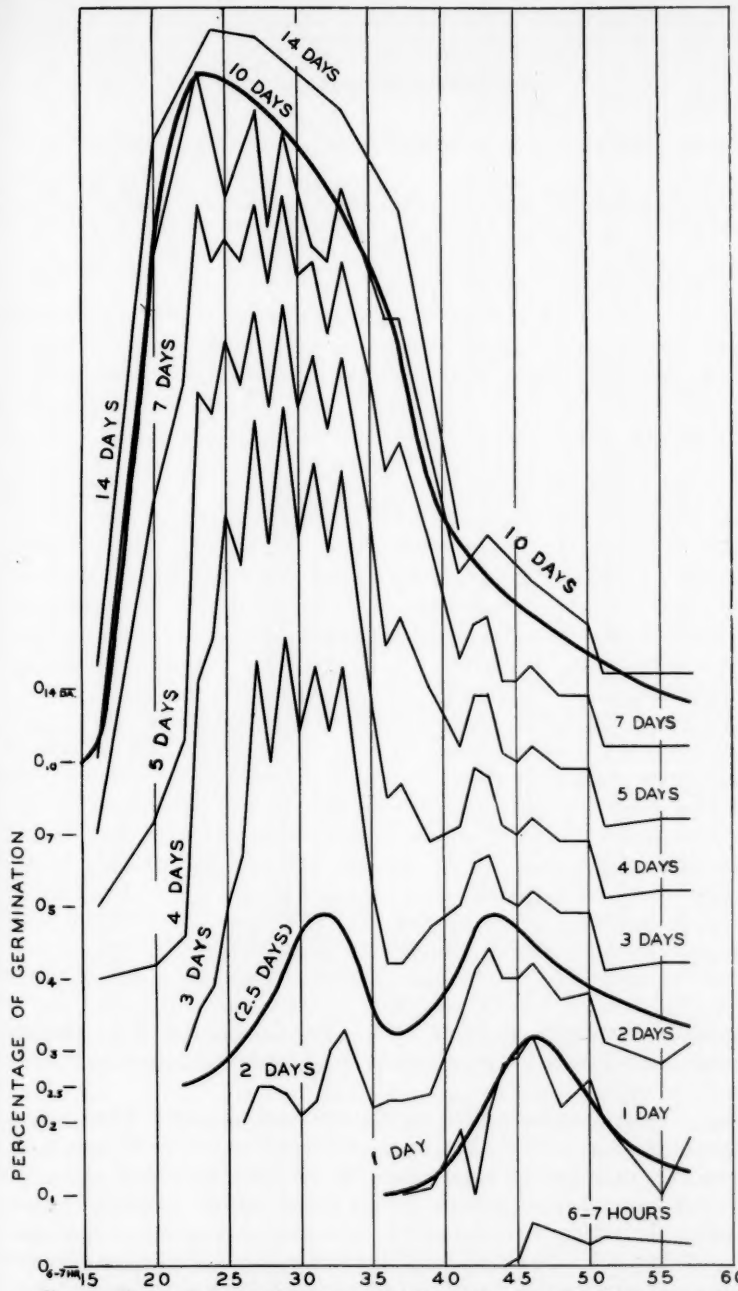

FIG. 1. Temperature-germination graphs for pitch pine seed, lot 1, for 6–7 hours, and for 1, 2, 3, 4, 5, 7, 10 and 14 days of incubation. The broad lines are smoothed graphs for the 1-day and 10-day periods and an interpolated graph for a period of incubation of about 2.5 days. Positions of the several different base lines are shown at the left, the subscript numerals indicating the respective lengths of incubation period.

TABLE II

MEAN PERCENTAGE OF GERMINATION (GERMINATIVE-ENERGY INDICES) OF SEEDS OF PITCH PINE, LOT 1, IN STANDARD CULTURE, FOR MAINTAINED TEMPERATURES BETWEEN 16° AND 57° AND FOR INCUBATION PERIODS OF FROM 6 OR 7 HOURS TO 14 DAYS. (THE NUMBER OF CULTURES ON WHICH EACH MEAN IS BASED IS SHOWN BY A SUBSCRIPT NUMERAL)

MAINTAINED TEMP.	PERCENTAGE OF SEEDS GERMINATED AT END OF								
Deg. C.	6-7-HR. PERIOD	1-DAY PERIOD	2-DAY PERIOD	3-DAY PERIOD	4-DAY PERIOD	5-DAY PERIOD	7-DAY PERIOD	10-DAY PERIOD	14-DAY PERIOD
16	—	0_5	0_5	0_5	0_5	0_5	0_5	1_4	3_2
20	0_3	0_{10}	0_{10}	0_{10}	2_{10}	12_{10}	46_{10}	70_6	76_2
22	—	0_2	0_2	0_2	6_2	23_2	62_2	—	—
23	0_1	0_4	0_4	6_4	41_4	71_4	87_4	95_2	—
24	0_2	0_6	0_6	9_6	47_6	68_6	79_6	87_3	91_2
25	0_1	0_6	0_6	20_3	64_3	78_3	82_3	78_1	—
26	0_1	0_2	0_2	27_2	57_2	72_2	79_2	—	—
27	—	0_5	5_5	54_5	77_5	82_5	87_5	90_4	90_2
28	0_2	0_7	5_7	40_4	60_4	69_4	76_4	74_1	—
29	—	0_2	4_2	57_2	79_2	83_2	88_2	87_1	—
30	0_1	0_1	1_1	44_1	61_1	69_1	77_1	—	—
31	0_1	0_3	3_3	53_3	71_3	76_3	79_3	71_1	—
32	0_2	0_2	10_2	44_2	59_2	66_2	69_2	69_1	—
33	—	0_{10}	13_{10}	53_7	70_7	76_7	79_7	79_5	80_2
35	—	0_2	2_2	22_2	38_2	53_2	63_2	—	—
36	0_3	0_9	3_9	12_8	25_8	36_8	50_6	61_3	—
37	—	0_5	3_5	12_5	27_5	40_5	54_6	61_4	66_2
39	0_1	1_1	4_1	17_1	19_1	30_1	41_1	—	—
41	0_4	9_{15}	15_{13}	20_{10}	21_{10}	22_{10}	24_{10}	26_5	22_1
42	—	0_1	21_1	26_1	29_1	29_1	29_1	—	—
43	0_1	11_1	24_1	27_1	28_1	29_1	30_1	31_1	—
44	0_1	18_1	20_1	21_1	21_1	21_1	21_1	—	—
45	1_2	19_3	20_3	20_3	20_3	20_3	21_3	—	—
46	6_2	22_2	22_2	22_2	22_2	22_2	23_2	25_1	—
48	—	12_1	17_1	19_1	19_1	19_1	19_1	—	—
50	3_2	16_2	18_2	19_2	19_2	19_2	19_2	19_1	—
51	4_2	10_4	11_4	11_3	11_3	11_3	12_3	12_1	—
55	—	0_1	8_1	12_1	12_1	12_1	12_1	—	—
57	3_1	8_1	11_1	12_1	12_1	12_1	12_1	12_1	—

maintained temperature employed (57°). For this lot of seed the absolute maximum temperature for germination was therefore surely considerably above 57°.

The minimal temperature for germination is shown to have been about 44° for the shortest period. For more prolonged incubation the minimum is shown to be progressively lower; about 38° for the 1-day period, about 26° for the 2-day period, and below 15° for the 14-day period. With still longer incubation the minimum would surely have appeared as well below, perhaps far below, 15°. To establish the absolute minimal temperature for the occurrence of germination in this lot of seed and for the background con-

ditions of these tests it would have been necessary to continue incubation much longer than fourteen days.

These observations on the temperature minimum emphasize at once the great importance of the duration factor in the consideration of this cardinal temperature. With seeds that germinate slowly it might be necessary to continue an experiment for many years before the absolute minimal temperature for germination could be ascertained. What percentage of the seeds of a lot can germinate at the absolute minimal temperature is another question, for answering which very prolonged incubation of the cultures would also be necessary.

Such considerations as these have been appreciated in a general way by those who have discussed minimal and maximal temperatures for physiological, and indeed for chemical and physical, processes, and it has frequently been noted that these two cardinal temperature values are usually capable of being ascertained only approximately. It may be remarked, however, that the maximum may always be stated as *above* a certain temperature (the highest temperature at which any germination occurred, for example), while the minimum may always be stated as *below* a certain temperature (the lowest at which the process referred to was observed).

With regard to optimal temperatures the graphs of figure 1 are especially interesting. For the shortest of the periods the optimum was only a degree or so above the corresponding minimum, being about 46° (6 per cent.). It was still about 46° (22 per cent.) for the 1-day period, but was somewhat lower, between 42° and 46° (21–24 per cent.), for the 2-day period. A second, and lower, optimum is clearly shown, however, for this same 2-day period of incubation, about 32°–33° (10–13 per cent.), and an intermediate range of maintained temperatures between the two optima (from about 35° to about 39°) gave remarkably low percentages of germination (2–4 per cent.). For the 3-day and 4-day incubation periods the high-temperature optimum (26–27 and 28–29 per cent., respectively) was about 42°–43°, but it had nearly disappeared for the 4-day period and is not evident for the longer periods represented in figure 1. The reason for the disappearance of the high-temperature optimum is clearly due to the fact that relatively few additional seeds germinated at about 42°–43° after the end of the 3-day period, while larger numbers of additional seeds germinated at lower temperatures. The depression in the graph between the two optimal temperatures is progressively less pronounced for the 3-day and 4-day periods, and it fails to appear in the graphs for the 5-day and longer periods of incubation.

The second or low-temperature optimum (32°–33°), shown as the smaller of the two in regard to percentage of germination for the 2-day period, is

represented by the highest percentage values on the graphs for the 3-day and longer periods. For the 5-day and longer periods, moreover, it is the only optimum and the graphs for these periods resemble, in form, many published graphs for the maintained-temperature relations of physiological processes.

For the 3-day period the low-temperature optimum (the one that appears as the only optimum for the longer periods, as just noted) is an optimal range, from about 27° to about 33° (40–57 per cent.); for the 4-day period this range is from about 25° to about 33° (57–79 per cent.); and for the 5-day and 7-day periods the optimal range (66–83 and 69–88 per cent.) is about the same as for the 4-day period. For the 10-day and 14-day periods the optimal temperature range appears to be narrower and located somewhat lower on the thermometer scale; for the longest incubation period represented, this range is from about 24° (91 per cent.) to about 27° (90 per cent.).

From these observations on the optimal temperatures it is very evident that the length of the period of incubation is a very important variable in the determination of the magnitude of the optimum and the characteristics of its range. The specification of this duration factor is even more essential in this connection than it is with regard to the temperature maximum and temperature minimum. As was emphasized by LEHENBAUER (**11**) and by FAWCETT (**6**), the magnitude of the temperature optimum for a process may, in general, be expected to vary considerably with the time factor, and statements as to temperature optima must usually be only vague and relatively unsatisfactory unless the length of the period of exposure is also given.

The importance of the length of the incubation period in the discussion of the characteristics of the temperature optimum for the occurrence of germination in this lot of seed is especially well shown when we consider the remarkable appearance of the double optimum for the 2-day and 3-day periods. It is evident that if these germination tests had been made with only 1-day, or with only 5-day or longer incubation periods, or if the periods used had been of several lengths but in every case less than 1 day or more than 4 days, no double temperature optimum would have been apparent from the numerical results and their graphs.

The three smoothed, broad-line graphs of figure 1 remain to be mentioned. The lowest of these is simply the smoothed graph for the 1-day period and the highest is the smoothed graph for the 10-day period. These give generalized pictures of the forms of the temperature-germination graphs for the short period (when the high-temperature optimum appears alone) and for the long period (when the low-temperature optimum is

alone evident). As the eye moves upward over the figure a continuous transition of the graph form, with progressively longer periods of incubation, is clearly indicated by the intervening narrow-line graphs, which represent the results of actual experiment. We may say that the lowest broad-line graph becomes gradually transformed as the incubation period is longer and longer, until it finally takes the form of the highest broad-line graph. In this continuous transformation the graph must pass through an intermediate form having two maxima with equal ordinates, each graph maximum representing one of the two temperature optima for germination. That particular stage in the graph transformation here envisaged is repre-

LENGTH OF PERIOD	MAXIMUM	HIGH-TEMPERATURE OPTIMUM (LARGE VALUES)	INTERMEDIATE TEMPERATURE (SMALL VALUES)	LOW-TEMPERATURE OPTIMUM (LARGE VALUES)	MINIMUM
6–7 hr.	57° +	46° (6 p. c.)	———	———	44°
1 da.	57° +	46° (22 p. c.)	———	———	38°
2 da.	57° +	42°–46° (21–24 p. c.)	35°–39° (2–4 p. c.)	32°–33° (10–13 p. c.)	26°
2.5 da.	**57° +**	**43.5° (24 p. c.)**	**36°–37° (7 p. c.)**	**31.5° (24 p. c.)**	.....
3 da.	57° +	42°–43° (26–27 p. c.)	36°–37° (12 p. c.)	27°–33° (40–57 p. c.)	22°
4 da.	57° +	42°–43° (28–29 p. c.)	39° (19 p. c.)	25°–33° (57–79 p. c.)	16°
5 da.	57° +	———	———	23°–33° (66–83 p. c.)	16°
7 da.	57° +	———	———	23°–33° (69–88 p. c.)	16°
10 da.	57° +	———	———	23°–29° (74–95 p. c.)	15°
14 da.	57° +	———	———	24°–27° (90–91 p. c.)	15°–

sented by the intermediate broad-line graph of the figure. This is not a smoothed graph for any series of experimental data actually available; it is a hypothetical graph, interpolated between the experimental graph for the 2-day period and that for the 3-day period, and it represents, as its parenthetical label indicates, an incubation period of approximately 2.5 days.

If the period of incubation represented by this interpolated graph had been employed in testing this lot of pitch pine seed the results would have been very striking indeed. For maintained temperatures about 31.5° and about 43.5° the percentage of germination would have been about 24, while it would have been only about 7 for a maintained temperature of about 36.5°.

To summarize the transition in graph form from that for the 6–7-hour period to that for the 14-day period, the cardinal features of the several graphs of figure 1 are tabulated, on p. 391. The interpolated data (for a 2.5-day period) are shown in black-face type.

The maximal temperature is above 57°. The minimal temperature recedes from about 44° (6–7-hr. period) to below 15° (14-day period). The high-temperature optimum recedes from about 46° (6–7-hr. period) to about 42° (4-day period) and then vanishes. The low-temperature optimum recedes from about 32° (2-day period) to about 25° (14-day period). The intermediate temperature, characterized by small percentage values, lies between 35° and 39°, being evident only for periods of from 2 to 4 days.

Special experiments with pitch pine seed, lot 1

Several special experiments with pitch pine seed of lot 1 were carried out with preliminary soaking in distilled water instead of in "Semesan" solution, standard procedure being otherwise employed. The results of these are shown by the graphs of figure 2, which show temperature optima for about 32° and about 41°, for incubation periods of 2 and 3 days, respectively. For the 10-day period a single optimum is shown as the temperature range from below 20° to about 32° (with germination percentages of

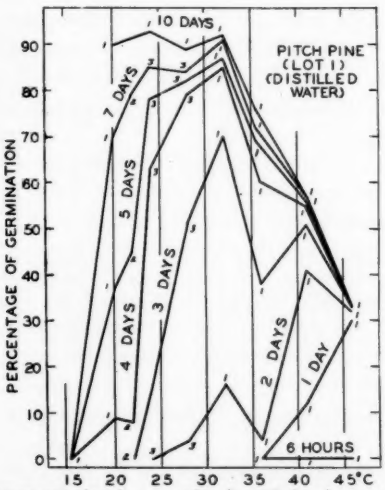

Fig. 2. Temperature-germination graphs for pitch pine seed, lot 1, preliminarily soaked in distilled water instead of "Semesan" solution, for 1, 2, 3, 4, 5, 7 and 10 days of incubation. No germination in 6-hour period. Numerals adjacent to points each indicate number of tests on which corresponding percentage value is based.

from 89 to 93). The temperature maximum is shown as at least above 46° and the minimum appears as about 36° for the 1-day period, about 24° for the 2-day period, about 22° for the 3-day period, and about 15° for the 4-day, 5-day, and 7-day periods. The numerals near the points on the graphs indicate how many cultures were used in each case.

A special experiment in which pitch pine seed of lot 1 was used without preliminary soaking at all, the dry seeds being distributed on the agar plates while the other details of the procedure were standard, showed for a 3-day incubation period one optimum about 30° (42 per cent.) and another for the temperature range from about 40° to about 46° (34 per cent.).

The double temperature optimum exhibited by the standard cultures of lot 1 of pitch pine seed is therefore not to be considered as related specially to the standard preliminary soaking in the antiseptic solution.

It seems clear that the recession of the minimal temperature with prolonged incubation and also the appearance of two separate optimal temperatures with intermediate lengths of the incubation period are related to an innate variability in the lot of seed. Apparently a sample of lot 1 of pitch pine consisted of several groups or categories of seeds, separable by experimentation of the type used in these studies. Turning again to the graphs of figure 1, it is evident that some of the seeds of each sample were capable of germination with an incubation period of 6 or 7 hours while some seeds failed to germinate at any temperature even with a 14-day period of incubation. If we regard the several cultures, of one hundred seeds each, as representative samples of the entire lot, about 20 per cent. of the lot were apparently capable of germination in a single day at a maintained temperature of 45°–46°, while none were able to germinate in a period of this length at any maintained temperature below 37°. With prolonged incubation (10–14 days) only about 30 per cent. of the seeds could germinate at the high-temperature optimum (42°–46°), but at the low-temperature optimum (24°–25°) 90 per cent. of them germinated in the longest incubation period. Most of the seeds characterized by capacity to germinate at the higher temperatures were also characterized by capacity to germinate *quickly* at those temperatures, but these same seeds were apparently capable of germination at the low-temperature optimum if incubation was sufficiently prolonged.

The seeds of this lot 1 may be tentatively considered as roughly grouped in three physiological categories according to their germinative capacity in regard to maintained temperature, as shown for the 14-day period of incubation: (A) those capable of germination at about 25° but not capable of germination at about 43°, (A′) those capable of germination at about 43° as well as at about 25° and (B) those not shown as viable at any maintained

temperature. The categories are not sharply distinguished but it appears that about 65 per cent. of this lot of seed belonged to the first category (A), about 25 per cent. to the second category (A′) and about 10 per cent. to the third category (B). With the third of these hypothetical groups we need not be concerned in the present connection, but the first two groups (A and A′) may be studied somewhat more closely by means of some available experimental data.

Many seeds that had germinated at 50° remained alive but grew only very slowly for as long as 10 days when kept at that temperature. If seeds that had germinated at about 50° were transferred, shortly after germination, to a temperature about 31° none of the seedlings grew well at the lower temperature and some were apparently dead at the time of transfer. Seeds that had germinated at about 43° and had then been immediately transferred to 31° showed seedling growth for 10 days, but the seedlings were apparently unhealthy. On the other hand, seedlings that had been produced at 31° and had then been transferred to 57° were dead within 8 days. In these special tests, the only seedlings that were growing well after 8 or 10 days were those that had been produced at about 31° and had been kept at that temperature.

These results indicate that, although about 25 per cent. (group A′) of the seeds of this lot of pitch pine were capable of germination at the high-temperature optimum, the resulting seedlings failed to develop satisfactorily with such a high temperature and were, indeed, likely to grow very poorly even at lower temperatures.

Other transfers were made from one maintained temperature to another to gain evidence as to whether the higher temperatures had killed the seeds that had failed to germinate quickly; that is, whether seeds of category A were killed by the high temperatures that produced the prompt germination of those of category A′. Cultures that had been exposed for 2 days or more to a temperature of 45° or 51° (in which, respectively, about 20 per cent. or about 10 per cent. of the seeds had germinated at the higher temperature) failed to show any additional germination in a 7-day period after they had been transferred to a temperature of 25° or 30°. In a 100-seed culture transferred from 45°, of which 20 per cent. of the seeds had germinated in 25 hours, 12 additional seeds had germinated at the end of the following 7-day period at 25°. In a culture transferred from 51°, of which 10 per cent. of the seeds had germinated in 25 hours, only one additional seed germinated in the subsequent 7-day period at 25°. Of seeds that had been first incubated at a temperature of 41° for 7 or 9 days (in which time about 15 per cent. or about 20 per cent. of them had germinated), only one or two more germinated at 25° in the next seven days.

Cultures that had been incubated at 36° for 7 days (with germination of about 50 per cent.) or for 10 days (with a somewhat greater percentage of germination) were transferred to 25°, after which many additional seeds germinated, the total percentage of germination 7 days after the change being 80 or 85, about as great as was shown by corresponding cultures transferred from a temperature of 33° to one of 25°. Cultures initially incubated at 37° for from 11 to 15 days showed a slight acceleration in the rate at which additional germination occurred after they had been transferred to a temperature of 25°.

Cultures initially incubated for 7 or 10 days at 25°, 29° or 33° continued to show additional germination after being transferred to 25°. Cultures initially incubated at 16° for 15 days, with a germination percentage of only about 5, showed total percentages of 75 or 95, 3 days after transfer to 25° or 30°.

From the results of these special experiments it appears that when pitch pine seeds of this lot, in standard cultures, were incubated at a high maintained temperature (about 45°–50°) for more than about 2 days, those that had not already germinated in that time at the high temperature had lost their capacity for germination, even at a temperature of about 25°. On the other hand, when seeds that had failed to germinate in 15 days at a low temperature (16°) were transferred to a temperature of 25° or 30°, germination was very prompt and general, and the total percentage of germination at the end of an additional 7 days was about 90. It is therefore apparent that the seeds of category A (capable of germination at 25° but not at 43°) were generally killed by incubation at the higher temperature for a period long enough to produce germination, at that temperature, of the other viable seeds of the same lot (category A′). Seeds capable of germination about the high-temperature optimum germinated there very quickly but produced apparently unhealthy seedlings. These seedlings died if kept at the temperature that produced them and they developed only slowly when transferred to the low-temperature optimum shortly after they had germinated at the high-temperature optimum.

It is of course conceivable that seeds capable of germinating at the higher optimal temperatures might be able to germinate at even higher temperatures and perhaps even more quickly. If that were true a triple optimal temperature for the occurrence of germination might appear. In the present study no temperatures above 57° were tested.

Other lots of pitch pine seed

Graphs for the results given by standard cultures of lots 2, 3, 4 and 5 of pitch pine seed are shown in figures 3 to 6, which are plotted in a manner similar to that adopted for the graphs of figures 1 and 2. The number

of tests (cultures) upon which each percentage value is based is shown in each case by a numeral adjacent to the corresponding point on the graph. The numbers are small in all cases and these experimental results are therefore less well established than are those for the pitch pine seed of lot 1.

For lot 2 (fig. 3) the percentage values are markedly lower than the

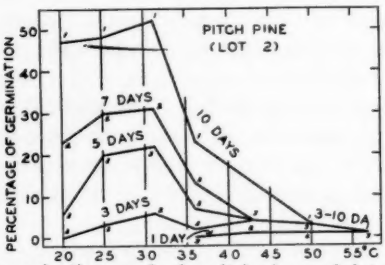

FIG. 3. Temperature-germination graphs for pitch pine seed, lot 2, for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

corresponding indices for lot 1, for all of the maintained temperatures tested and for all the different lengths of incubation period for which data are available. A high-temperature optimum is indicated for the 1-day period and a double optimum (about 31° and about 43°) appears for the 3-day period. The maximum is shown as about 57°. The minimum is perhaps about 36° for the 1-day period and about 20° for the 3-day period. The low-temperature optimum is the range from about 25° to about 31° for the 5-day and for the 7-day periods for which it is the only optimum shown. For the 10-day period this range appears as from about 20° to about 31°.

For lot 3 (fig. 4) no indication of a double optimum appears. The

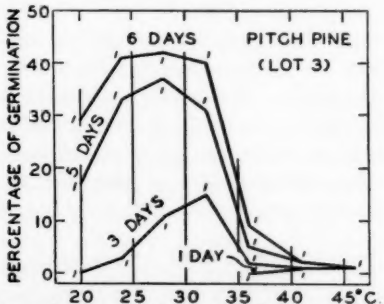

FIG. 4. Temperature-germination graphs for pitch pine seed, lot 3, for 1, 3, 5 and 6 days of incubation. Each percentage is based on but a single culture.

maximum temperature is indicated as above 45°. The minimum is about 36° for the 1-day period and about 20° for the 3-day period. The single (low-temperature) optimum is shown as about 32° for the 3-day period and it appears to be the range from about 24° to about 32° for the 5-day and 6-day periods.

For lot 4 (fig. 5) a high-temperature optimum is indicated for the 1-day

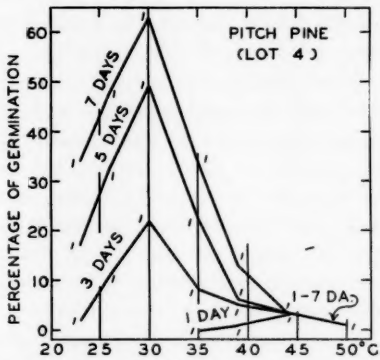

FIG. 5. Temperature-germination graphs for pitch pine seed, lot 4, for 1, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

period (about 44°) and a low-temperature optimum appears about 30° for the 3-day, 5-day and 7-day periods, but no double optimum is actually shown. The maximum appears as above 50° and the minimum is shown about 35° for the 1-day period and below 23° for the 3-day period.

For lot 5 (fig. 6) a high-temperature optimum about 45° or above is

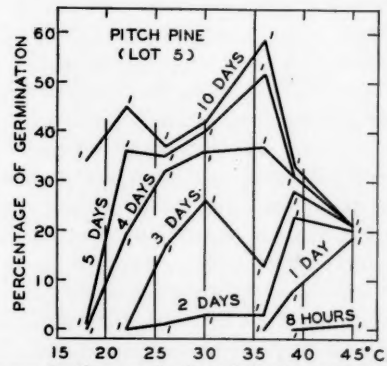

FIG. 6. Temperature-germination graphs for pitch pine seed, lot 5, for 8 hours and for 1, 2, 3, 4, 5 and 10 days of incubation. Each percentage is based on but a single culture.

suggested for incubation periods of 8 hours and 1 day and a similar optimum about 39° is shown for periods of 2 and 3 days. A low-temperature optimum (about 30°) appears with the other for the 3-day period, thus giving a double optimum for that period, with two nearly equal germinative-energy indices. For the longer periods of incubation that were tested the single optimum appears about 36°. The maximum is not indicated (excepting that it is shown as above 45°) and the minimum appears about 39° for the 8-hour period, about 36° for the 1-day period, about 22° for the 2-day and 3-day periods, about 18° for the 4-day period and below 18° for the 5-day and 10-day periods.

In general, the results of these standard tests with lots 2, 3, 4 and 5 of pitch pine seed indicate that lot 1 was peculiar in several respects, but they show that the double optimum so clearly established for seed of that lot may be expected from tests of other lots of this species, though exceptions are to be expected also. It appears that this phenomenon of a double optimum is related to differences (variability) in the internal conditions or physiological characteristics of the seeds of each lot for which it was exhibited. These differences and their distribution in the lot must have acted together with the environmental background conditions of the standard treatment. It does not seem desirable to attempt here to make suggestions as to precise and detailed reasons for the differences between these lots of pitch pine seed, although a number of apparently plausible hypotheses might be imagined. With the meager data at hand it is best to avoid any attempt in that direction and to say simply that the double optimum was shown for some lots of pitch pine seed in standard cultures for relatively short periods of incubation, while it failed to become evident in similar tests of other lots of seed of the same species. If a little deeper but very general analysis is desired, it seems to be safe to say: (1) that the individual seeds of each lot differed among themselves in their capacity to germinate under the several different sets of experimental conditions used in the tests, each set of conditions embracing, besides the standard background complex, one particular maintained temperature and one length of the incubation period; (2) that the seeds of any lot may be conveniently classified in a number of physiological categories according to their individual capacities for germination under the influence of the several experimental conditional complexes; and (3) that each lot was characterized by its own set of proportions of the seeds belonging to the several categories. The graphs of germinative energy as related to the duration of incubation and to temperature furnish pictures of the ways in which the lots differed in statistical make-up. According to these pictures the several lots might themselves be grouped in lot categories.

Seeds of other coniferous trees

Results of some less extensive experiments with lots of seed of the other coniferous trees dealt with in this study are summarized by the graphs of figures 7–15. The experimental procedure was standard for all but loblolly pine (fig. 9), longleaf pine (fig. 10), western yellow pine (fig. 12) and one series of Engelmann spruce (fig. 13, broken lines). In the case of loblolly pine only 50 seeds were used per culture and the preliminary soaking was in distilled water. In the case of longleaf pine only 25 seeds were used per culture, the procedure being otherwise standard. For Engelmann spruce, the series represented by broken lines in figure 13 differed from the standard only in that the preliminary soaking was in distilled water. The cultures of western yellow pine were standard excepting that only 50 seeds were used for each.

The experimental data for these nine species are in every case much less extensive than those for pitch pine seed, lot 1, and they are to be regarded as not very significant; but several of these series are clear in their indications concerning the double optimal temperature for germination. The graphs for these series are constructed like those previously presented and do not require special discussion, but the following notes on them are of interest.

For red pine (fig. 7) two temperature optima (about 24° and about 36°)

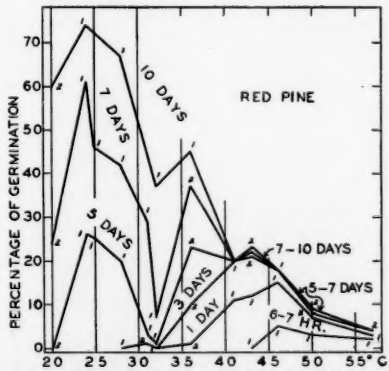

Fig. 7. Temperature-germination graphs for seeds of red pine, for 6–7 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

are evident for incubation periods of 5, 7 and 10 days. For the two shorter periods tested (6–7 hours and 1 day) only a single optimum is shown (about 46°).

For lodgepole pine (fig. 8) two optima (about 24° and about 40°) appear for incubation periods of 3, 5, 7 and 10 days, and but one optimum

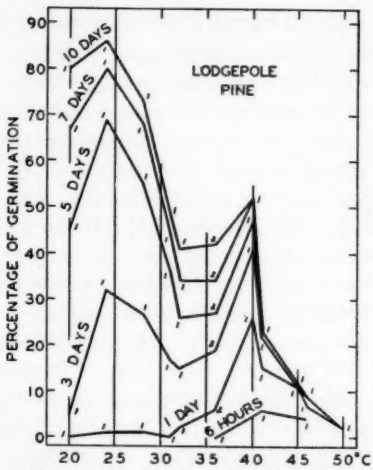

FIG. 8. Temperature-germination graphs for seeds of lodgepole pine, for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Numerals adjacent to points as in fig. 2.

(about 40°–41°) is indicated for the two shorter periods (6 hours and 1 day).

For loblolly pine (fig. 9) two optima (about 26° and about 40°) appear

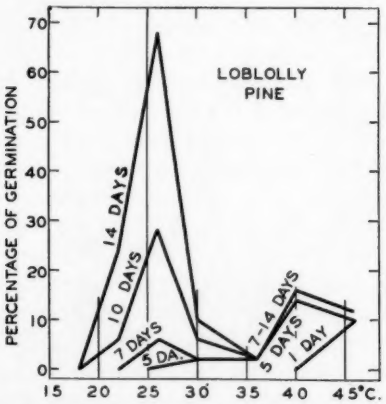

FIG. 9. Temperature-germination graphs for seeds of loblolly pine (50 seeds to the culture and preliminary soaking in distilled water instead of antiseptic solution), for 6.5 hours and for 1, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

for 7, 10 and 14 days. A single optimum is indicated for the 1-day period as above 46°, while for the 5-day period it appears to be about 40°.

For longleaf pine (fig. 10) two optima (apparently below 18° and above 46°) are indicated for incubation periods of 5, 7 and 10 days. For periods of 1 day and 3 days a single optimum is indicated as above 46°.

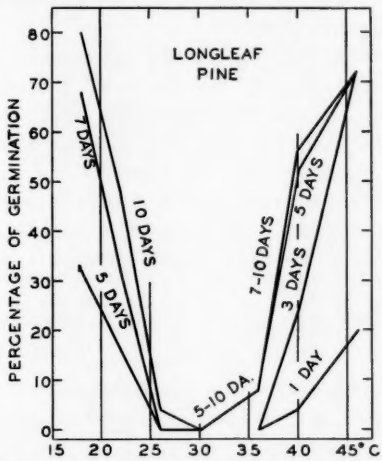

FIG. 10. Temperature-germination graphs for seeds of longleaf pine (25 seeds per culture), for 6 hours and for 1, 3, 5, 7 and 10 days of incubation. Each percentage is based on but a single culture.

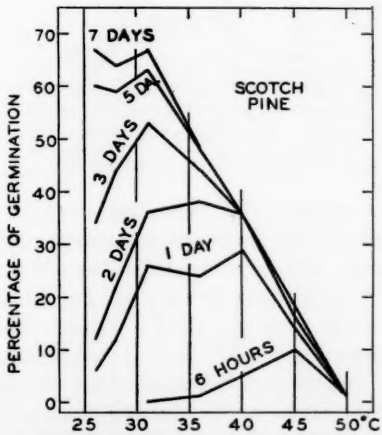

FIG. 11. Temperature-germination graphs for seeds of Scotch pine, for 6 hours and for 1, 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

It was not until the tenth day of incubation that any seed germinated with a maintained temperature of 26°, and no germination had yet occurred at 30° when the experiment was discontinued after the cultures had been incubated 10 days. After the seventh day no additional seeds germinated with any temperature above 26°.

For Scotch pine (fig. 11) only a single optimum is indicated; about 45° for the 6-hour period, about 40° for the 1-day period, about 36° for the 2-day period, about 31° for the 3-day and 5-day periods and between 26° and 31° for the 7-day period. Two optima (about 31° and about 40°) are perhaps suggested for the 1-day period. A period of about 18 hours might have shown two optima, if such a period had been tested. It may be noted that SCHIMPER (**20**, page 46) quotes DETMER as stating that the maximal temperature for the germination of seed of Scotch pine is 34°, while the optimal temperature is 27°.

For western yellow pine (fig. 12) only a single optimum is shown;

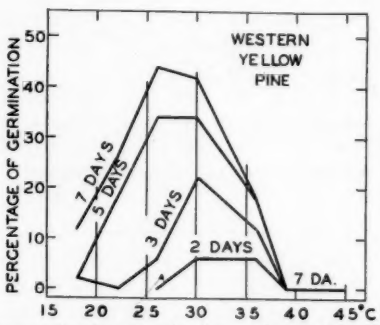

FIG. 12. Temperature-germination graphs for seeds of western yellow pine (50 seeds per culture), for 2, 3, 5 and 7 days of incubation. Each percentage is based on but a single culture.

between 30° and 36° for the 2-day period, about 30° for the 3-day period and between 26° and 30° for the 5-day and 7-day periods. It is possible that two optima might have appeared for the 2-day period if suitable maintained temperatures between 30° and 36° had been tested.

For Engelmann spruce (fig. 13) there were two series, for one of which the cultures had standard treatment while the seeds used in the other series had been soaked in distilled water instead of the standard antiseptic solution. In figure 13 the graphs for the standard tests are full lines and those for the special tests are broken lines. Two optima are clearly indicated (about 26° and about 40°) for periods of 3 and 5 days, also (about 26° and about 36°) for the 7-day period. A single high-temperature optimum

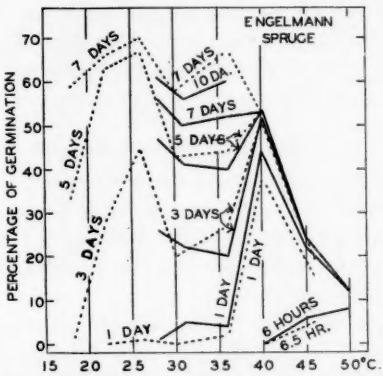

FIG. 13. Temperature-germination graphs for seeds of Engelmann spruce for 6–6.5 hours and for 1, 3, 5, 7 and 10 days of incubation. Solid lines represent standard cultures and broken lines represent cultures of seeds preliminarily soaked in distilled water instead of antiseptic solution. Each percentage is based on but a single culture.

(about 40°) appears for the 1-day period and the same is true (perhaps about 50° or even higher) for the period of 6 or 6.5 hours. As in the case of lot 1 of pitch pine seed, a satisfactory general agreement is shown between the results of the standard cultures and those of the tests with water-soaked seeds.

For white spruce (fig. 14) only a single optimum is indicated; about

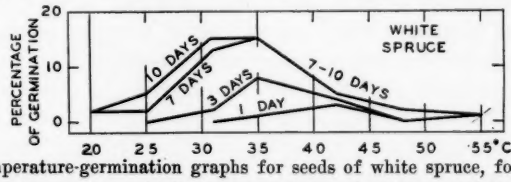

FIG. 14. Temperature-germination graphs for seeds of white spruce, for 1, 3, 7 and 10 days of incubation. Each percentage is based on but a single culture.

42° for the 1-day period, about 35° for the 3-day and 7-day periods, and between 30° and 35° for the 10-day period.

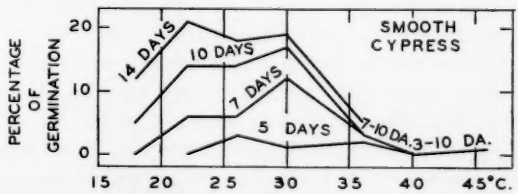

FIG. 15. Temperature-germination graphs for seeds of smooth cypress, for 3, 5, 7, 10 and 14 days of incubation. Each percentage is based on but a single culture.

For smooth cypress (fig. 15) two optima (about 26° and about 36°) are suggested for the 5-day period, but only a single optimum (about 30°) is indicated for the 7-day and 10-day periods, while two optima (about 22° and about 30°) are suggested for the 14-day period.

Résumé of results on temperature optima, and additional notes

For convenience of reference and for comparison the main indications about optimal temperatures for the occurrence of germination in the several lots of seed, as set forth in the preceding sections of this paper and by the graphs of figures 1 to 15, are brought together in table III, the arrangement of which is self-explanatory.

There appears to be no doubt that the double optimum observed for lot 1 of pitch pine seeds is to be expected not only for other lots of seed of that species, but also for lots of seed of five other coniferous trees (red pine, lodgepole pine, loblolly pine, longleaf pine and Engelmann spruce). This expectation is suggested for Scotch pine and smooth cypress also. In some cases the double optimum is indicated for a wide range of lengths of the incubation period while in other cases it is confined to certain intermediate periods of incubation if these are neither too long nor too short.

In connection with the remarkably high maximal temperatures indicated for most of the lots of seed dealt with in this paper (50° or above, and apparently as high as 60° for pitch pine seed of lot 1), it may be remarked that seeds in nature may be temporarily subjected to temperatures above 60° for limited periods of time, as during hot sunny periods, if they lie on or near the soil surface. This may well be of frequent occurrence in the case of some species of pine that grow in areas subjected to high temperature and intense sunshine. For example, Bates (**1**) observed occasional temperatures of 66° C. about 0.5 cm. below the soil surface on south slopes in the central Rocky Mountain region, and Toumey and Neethling (**23**) record an exreme soil-surface temperature of 67° C. for New Hampshire. When such high soil temperatures occur they are apt to be confined to short periods (perhaps less than 12 hours) but they may be repeated for several days in succession or at longer intervals. It seems doubtful that moisture conditions suitable for seed germination are apt to be present at or near the soil surface when these very high temperatures occur.

In nature germinating seeds are almost always subjected to fluctuating temperature rather than to maintained temperature and the possible ecological bearing of the influence of temperature fluctuations on plant activities can not be approached seriously until much laboratory experimentation and precise analysis have been devoted to the general physiological problem of the influence of fluctuations in temperature as these affect the funda-

TABLE III

SUMMARY OF DATA ON OPTIMAL TEMPERATURES FOR THE OCCURRENCE OF GERMINATION IN THE SEVERAL LOTS OF SEEDS STUDIED, DERIVED FROM TABLE II AND FIGURES 1–14

KIND OF SEED AND TREATMENT	FIGURE NO.	LENGTH OF INCUBATION PERIOD	OPTIMAL TEMPERATURE		
			DOUBLE		SINGLE
			LOW	HIGH	
			Deg. C.	*Deg. C.*	*Deg. C.*
Pitch pine, lot 1 (standard cultures)	1	6–7 hr.	——	——	46
		1 da.	——	——	46
		2 da.	32–33	42–46	——
		3 da.	27–33	42–43	——
		4 da.	25–33	42–43	——
		5–7 da.	——	——	23–33
		10 da.	——	——	23–29
		14 da.	——	——	24–27
Pitch pine, lot 1 (soaked in distilled water)	2	1 da.	——	——	46
		2–3 da.	32	41	——
		4–5 da.	——	——	32
		7 da.	——	——	24–32
		10 da.	——	——	20–32
Pitch pine, lot 2 (standard cultures)	3	3 da.	31	43	——
		5–7 da.	——	——	25–31
		10 da.	——	——	20–31
Pitch pine, lot 3 (standard cultures)	4	3 da.	——	——	32
		5–6 da.	——	——	24–32
Pitch pine, lot 4 (standard cultures)	5	1 da	——	——	44
		3–7-da.	——	——	30
Pitch pine, lot 5 (standard cultures)	6	8 hr.	——	——	45
		1 da.	——	——	45
		2 da.	——	——	39
		3 da.	30	39	——
		4 da.	——	——	30–36
		5–10 da.	——	——	36
Red pine (standard cultures)	7	6–7 hr.	——	——	46
		3 da.	——	——	46
		5-10 da.	24	36	——
Lodgepole pine (standard cultures)	8	6 hr.	——	——	41
		1 da.	——	——	40
		3–10 da.	24	40	——
Loblolly pine* (soaked in distilled water)[a]	9	1 da.	——	——	46+
		5 da.	——	——	40
		7–14 da.	26	40	——
Longleaf pine* (standard cultures)[b]	10	1–3 da.	——	——	46+
		5–10 da.	18–	46+	——
Scotch pine* (standard cultures)	11	6 hr.	——	——	45
		1 da.	31(?)	40(?)	40(?)
		2 da.	——	——	36
		3–5 da.	——	——	31
		7 da.	——	——	26–31(?)

See next page for footnotes.

TABLE III—*Continued*

Kind of seed and treatment	Figure no.	Length of incubation period	Optimal temperature		
			Double		Single
			Low	High	
			Deg. C.	*Deg. C.*	*Deg. C.*
Western yellow pine (standard cultures)	12	2 da.	——	——	30–36
		3 da.	——	——	30
		5–7 da.	——	——	26–30
Engelmann spruce* (standard cultures)	13	6 hr.	——	——	50+
		1 da.	——	——	40
		3–7 da.	27–	40	——
Engelmann spruce* (soaked in distilled water)	13	6.5 hr.	——	——	46+
		1 da.	——	——	40
		3–5 da.	26	40	——
		7 da.	26	36	——
White spruce (standard cultures)	14	1 da.	——	——	42
		3–7 da.	——	——	35
		10 da.	——	——	31–35
Smooth cypress* (standard cultures)	15	5 da.	26(?)	36(?)	——
		7–10 da.	——	——	30
		14 da.	——	——	22(?)

[a] 50 seeds per culture.

[b] 25 seeds per culture.

* A plus or minus sign after a number indicates that the number is too small or too large, respectively. An interrogation point in parentheses after a number indicates that the magnitude is questionable.

mental plant processes. Such investigation must, in turn, wait for much further advance in our knowledge of the influences exerted on plants by maintained temperature, a subject that has hardly yet begun to receive attention. The whole subject of temperature influence upon organisms constitutes a practically uncultivated field, the problems of which become more complex with each small contribution. Very fundamental physiological research will be needed before these problems may be subjected to sufficiently searching analysis to permit of the planning of experimentation by which they may possibly be simplified and rendered directly approachable.

The phenomena of double temperature optima for the occurrence of seed germination disclosed by the results of the present study are of peculiar interest because of their novelty and they may prove to be of great importance in the study of physiological temperature relations. The possible occurrence of two maintained-temperature optima for germination in a lot of seed is established by the present report, but the relation of these phe-

nomena to plant species and to lots of seed other than those here dealt with, as well as to other influential environmental complexes than those here tested, remains to be taken up. Seed of some species may not exhibit these phenomena at all while that of other species may exhibit them in some lots of seed but not in every lot. The fact that a double temperature optimum for the occurrence of germination has not been discovered for a given kind or lot of seed does not, of course, preclude the possibility that this characteristic may be disclosed in other lots of the same sort of seed or in lots of other sorts. The great significance of the length of the incubation period has been emphasized in earlier sections, and experimentation by which the occurrence of double temperature optima is to be sought must therefore be planned to embrace a number of different lengths of period as well as a number of different maintained temperatures. And the values for both of these variables must be so chosen as to cover the logical field adequately. The lengths of incubation period employed should differ serially by relatively small steps and the same is true of the maintained temperatures used. The range of period length and that of temperature must of course be broad enough to allow the double optimum to become manifest if the lot of seed in question should possess the capacity for exhibiting it. Finally, the environmental background complex employed in such studies as are here suggested (including all influential external conditions excepting temperature and duration) must surely be highly important in the determination of the occurrence or non-occurrence of evidence of a double optimum in any series of tests. It may be surmised that water relations and oxygen relations might furnish limiting conditions in many instances and the mind staggers at the thought of the infinite array of other possibilities suggested by such expressions as chemical influences, and chemical stimulation.

From studies of very different types, double temperature optima have been reported by KOEPPEN (**9**), for elongation of shoots, and by YAMAHA (**25**), for certain cytological processes in root tips; and the data given by WIESNER (**24**) for rate of germination of fungus spores indicate that he also encountered this phenomenon. The results of these studies cannot, however, be profitably compared with those of the present study.

It may be remarked that the separation of the individuals of a lot of seed into physiological categories, as such a separation or sorting results from studies like the ones here considered, is likely to be related to the general vitality of the seeds, to their varying degree of vital capacity in other respects as well as with regard to viability. It seems probable that the process of respiration may offer useful clues in this connection. If comparative studies on respiration might be conducted concomitantly with thoroughgoing experimentation on germinative energy enlightening results might be expected.

Summary and conclusion

This paper presents the results of experimental studies on the germinative energy of lots of seed, as the measure of this lot characteristic is influenced by the maintained temperature and by the length of the period of incubation employed in the experimental tests. Five lots of seed of pitch pine (*Pinus rigida* Mill.) were studied and several lots of seed of other coniferous trees. The seeds were usually subjected to a preliminary soaking in an antiseptic solution (to retard development of fungi), after which they were regularly distributed on the surfaces of agar plates, each in a glass Petri dish. The cultures thus prepared were themselves distributed among the incubation chambers for each experiment series. Thirty-six different maintained temperatures were employed, ranging from 8° to 57° C. and varying for the most part by intervals of one degree, and temperature fluctuation was usually not more than a single degree above or below the mean for the given chamber. Observations were made at daily intervals or more frequently, the length of the shortest period of incubation being 6–7 hours while that of the longest was 14–15 days. A seed was considered as having germinated when its radicle distinctly protruded from the split seedcoat. The number of seeds that had germinated in each culture was recorded for each incubation period and this number was expressed as a percentage of the total number of seeds in the culture, this percentage value being taken as a numerical index of germinative energy for that sample of seeds for that period and temperature. Mean index values were computed by averaging the corresponding percentage values for all similarly treated cultures. For each lot of seed a series of mean indices was thus obtained for each length of incubation period, there being a single mean index for each of the different maintained temperatures tested for that length of period.

The variables dealt with were: (1) the kind of seed tested (species and lot), (2) the maintained temperature and (3) the length of the incubation period. Temperature-germination graphs present the results, abscissas representing temperatures while ordinates represent the corresponding mean germinative-energy indices. A separate graph is presented for each length of incubation period tested, for each lot of seed studied.

The main aim of these studies was to ascertain approximately for each lot of seed the optimal maintained temperature for the occurrence of germination, for each length of incubation period tested. Some attention was given to the other cardinal temperatures also (minimal and maximal) and to the manner in which the form of the temperature-germination graph alters with the length of the incubation period.

For any germination to occur among the seeds of the lot of pitch pine seed to which this study was mainly devoted, the maintained-temperature maximum appears to have been above 57°, perhaps as high as 60°. The minimal maintained temperature is shown as about 44° for the shortest incubation periods (6 or 7 hours) that gave any germination at all. For progressively longer incubation periods the minimal maintained temperature appears to have been progressively lower and a 14-day period of incubation gave a minimum below 15°.

For the shortest incubation periods (6–7 hours) that gave any germination in this lot of pitch pine seed, the optimal maintained temperature was near the minimum, about 46°. For long periods (4–14 days) the optimal temperature appears as a single optimal range extending from about 23° to about 33°, the upper limit of the range being progressively lower for longer periods. For incubation periods of intermediate length a *double optimum* is clearly and unmistakably shown by the experimental data, a high-temperature optimum (corresponding to the single optimum for the shortest periods) and a low-temperature optimum (corresponding to the single optimum for the longest periods). For a 3-day period of incubation these two optima are represented by the temperature ranges from 27° to 33° and from 42° to 43°, respectively. The temperature-germination graph for this intermediate length of period consequently shows two high regions with a pronounced depression between. If a hypothetical graph for an incubation period of about 2.5 days is constructed by interpolation, the germinative-energy index for the high-temperature optimum (43°–44°) becomes numerically the same (24 per cent.) as that for the low-temperature optimum (32°–33°). This means that, for a properly chosen length of incubation period this lot of seed should be expected to exhibit about the same high percentage of germination with maintained temperatures about 33° and about 43°, while the expected corresponding value for an intermediate temperature about 36°–37° is very much lower (about 7 per cent.).

The phenomenon of a double maintained-temperature optimum (two different optima) for the occurrence of germination in a lot of seed is here reported apparently for the first time. It is clearly shown for lot 1 of pitch pine seed and is indicated for lots 2 and 5, but was not evident for lots 3 and 4. It therefore appears to have characterized some, but not all, of the lots of pitch pine seed studied. Similar pairs of temperature optima are shown for single lots of seed of red pine (*Pinus resinosa* Ait.), lodgepole pine (*Pinus murrayana* Balf.), loblolly pine (*Pinus taeda* L.), longleaf pine (*Pinus palustris* Mill.) and Engelmann spruce (*Picea engelmanni* Engelm.), and they are suggested for Scotch pine (*Pinus sylvestris* L.) and smooth cypress (*Cupressus glabra* Sudw.). A single lot of rice seed (*Oryza*

sativa L.) was somewhat thoroughly studied in connection with the experimentation here reported, without the discovery of any indications of a double temperature optimum. It is possible that double optima may be found to characterize the temperature-germination relations of lots of many kinds of seed.

A lot of seed may be classified in groups or categories, according to the different proportions of its individuals that are capable of germination under the influence of different sets of temperature and duration conditions. For example, lot 1 of pitch pine seed, for which the most extensive data are available from this study, may be considered as having been made up of at least three different physiological forms or types: (A) seeds capable of germination in 14 days or less at a temperature about 25° but not at a temperature about 43°, (A′) seeds capable of germination at both of these maintained temperatures within 14 days or less, and (B) the rest of the lot, not capable of germination in 14 days at any temperature, with the background conditions that were employed. These three categories appear to represent, respectively, about 65, 25 and 10 per cent. of the lot. Seeds of category A were permanently injured when incubated for a day or two at about 43° and they failed to germinate subsequently when transferred to a temperature about 25°. Seeds of category A′ germinated very quickly at about 43° but they produced unhealthy seedlings, which grew but slowly even after being transferred to a temperature about 25°. The high-temperature optimum is thus seen to favor quick germination of the seeds of category A′, but apparently at the expense of subsequent vitality in the seedlings produced.

JOHNS HOPKINS UNIVERSITY,
BALTIMORE, MARYLAND.

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OSMOTIC PRESSURE, OSMOTIC VALUE, AND SUCTION TENSION

WILLIAM A. BECK

(WITH TWO FIGURES)

Introduction

The term osmotic pressure has been widely misapplied to various osmotic quantities in plant physiology, particularly before the year 1916.

In that year URSPRUNG (**45, 46, 47, 48, 49**) began a critical examination of the literature referring to osmotic quantities in plant cells, and set about giving precision to the term "osmotic pressure." He has coined some new terms for the osmotic quantities which are entirely different in concept and numerical value from "osmotic pressure." He furthermore devised ways and means of measuring the quantities which he defined.

For several years his reform in terminology has been generally accepted in Europe. The paper which the writer read for URSPRUNG (**44**) before the International Congress of Plant Scientists at Ithaca, New York, in 1926, was well received.

Even at this date the transactions of the Congress have not appeared in print, so that the ideas of URSPRUNG are not sufficiently clear to many in this country. It is to help clarify the thinking in this country that this paper has been written. Another motive was to show the relations and differences between the quantities which are most frequently confused, *i.e.*, osmotic pressure, osmotic value, and suction tension.

URSPRUNG's paper as it will appear in print will not indicate the original sources, since practically all of the studies in the domain of the newly-defined quantities were carried out in his laboratories. A larger bibliography is appended to this work than may seem necessary, in order to help those who wish to take up intensive work in this field.

Osmotic pressure

With keen insight VAN'T HOFF (**20**) perceived a relation between PFEFFER'S (**35**) remarkable osmotic experiments with plants and the properties of solutions. This discovery led to his theory of solutions, a theory which has come to be regarded as indispensable in physical chemistry, and in both plant and animal physiology.

As is well known, certain sensitive plants like *Mimosa* respond readily to stimuli. PFEFFER attempted to explain the responses of such plants, and

was able to show that certain cells of the pulvini decrease in volume during response, simultaneously exuding water into the intercellular spaces. After some time, the cells were able to absorb the water from the intercellular spaces again, so that the original volume was once more restored, and the tone was automatically reëstablished.

In an effort to measure the active forces which caused the cells to contract and expand, the contracted tissues were stretched, and the force necessary to cause the stretching was determined. The results showed that the active forces in the cells were very great. There remained no doubt that the pressure on the inner side of the cell wall was at least between two and four atmospheres, when the cell was in normal condition. The nature of the phenomenon seemed to be the same as that observed in the experiments with the osmometer of DUTROCHET, so that it was natural to suppose that the force exerted by the cell under tension was equal to the force which caused the water to enter the cell, and furthermore, that the equilibrant of this force was comparable to the hydrostatic pressure (or weight of the mercury when mercury was employed) in the osmometer. Just as the equilibrant force prevented the solution in the osmometer from taking in more water, so did the pressure exerted by the wall prevent more water from entering the cell.

The order of the figures obtained with the osmometer of DUTROCHET did not warrant the conclusion that the phenomenon could be explained by osmosis alone. By using the semipermeable membranes of TRAUBE the results obtained were more satisfactory, and were of the order expected. Some of the results are given in table I.

TABLE I

THE OSMOTIC PRESSURE OF SUCROSE

CONCENTRATION IN PER CENT.	OSMOTIC PRESSURE IN CM. OF MERCURY	RATIO OF PRESSURE TO PER CENT. CONCENTRATION
1	53.5	53.5
2	101.6	50.8
4	208.2	52.1
6	307.5	51.3

From the first two columns it appears at once that the equilibrant of the force which causes the water to enter, "osmotic pressure," is a function of the concentration. The coefficient expressing the relation of the pressure to the concentration (given in the last column) is particularly interesting, and is fairly constant. It indicates that there must be some relation be-

tween the number of molecules of the solute present and the potential force of water influx, which is measured by the equilibrant.

Other experiments showed that the pressure exhibited by the osmometer is a function of the temperature and the relation may be expressed by the formula:

$$p = n \times 0.652 \times (1 + \alpha t) \tag{1}$$

in which p is the pressure, n is the number of grams of the solute per 100 grams of solution, t the absolute temperature in centigrade degrees, and α a temperature constant determined experimentally.

This mathematical expression reminds one of the formula expressing the gas law. The analogy becomes more striking when it is noted that the accepted value of α is 0.00367 or 1/273.

VAN'T HOFF used the results of PFEFFER in an attempt to prove that the above formula is entirely analogous to the gas law, *i.e.*, the constant R which is given by the ratio $pv/273$ is the same in the formula expressing the gas law, and in the one expressing the law of osmotic pressure.

According to the gas law the constant R is give by

$$pv/273 = 0.0821$$

in which p is the pressure, v is the volume of the gas, and 273° is the absolute temperature at 0° centigrade. Particular attention is to be given to the fact that in the gas law the pressure is real and expressed, and the volume is the actual volume, while in the osmotic law the pressure is a potential pressure, and the volume is an ideal volume.

If we consider the volume of a given solution of definite concentration, as the volume which is required to contain a gram molecular equivalent of solute, and the pressure as the equilibrant of the force with which water is forced into the osmometer, we can calculate the value of r, which appears in the mathematical expression of the osmotic law. From PFEFFER's results p was found to be 0.652 for a 1 per cent. solution of sucrose at 0° C.; 100 cc. of normal sucrose solution would contain 34.2 grams. The volume of a 1 per cent. solution required to contain 34.2 grams of solute would be 34.2 times the unit volume (100 cc.). From this it follows that,

$$pv/273 = 0.652 \times 34.2/273 = 0.0817 = r \tag{2}$$

This example illustrates that the constant r which is used in deducing the osmotic pressure, is practically the same in numerical value, as the constant employed in the gas law.

When a solution is separated from the pure solvent by a semipermeable membrane, and a difference of the pressures (measured directly) exerted on the solution and solvent produces a condition of equilibrium (mechan-

ical), such that there is no tendency for the solvent to flow in either direction through the membrane, the difference in pressure is defined as the "osmotic pressure."

VAN'T HOFF based his discussion of solutions on the data of PFEFFER, which were the best to be had at the time. Since, MORSE and FRAZER (**32, 33**) obtained much more reliable results. MORSE showed that much better harmony and exactness resulted if the volume is expressed in terms of the solvent, rather than in terms of the solution. In table II a comparsion of the calculated results according to the two systems is given.

TABLE II

COMPARISON OF THE CALCULATED OSMOTIC PRESSURES

CONCENTRATION OF SUCROSE		OSMOTIC PRESSURE CALCULATED ACCORDING TO		OBSERVED OSMOTIC PRESSURE
WEIGHT NORMAL (MOLS. PER 1000 GM. OF WATER)	VOLUME NORMAL (MOLS. PER LITER OF SOLUTION)	MORSE AND FRAZER	VAN'T HOFF	
0.1	0.098	2.40	2.36	2.59
0.2	0.192	4.81	4.63	5.06
0.3	0.282	7.21	6.80	7.61
0.4	0.370	9.62	8.90	10.14
0.5	0.453	12.00	10.90	12.75
0.6	0.533	14.40	12.80	15.39
0.7	0.610	16.80	14.70	18.13
0.8	0.685	19.20	16.50	20.91
0.9	0.757	21.60	18.20	23.72
1.0	0.825	24.00	19.80	26.64

It is evident from the figures that some definite law governs the force with which the water enters the osmometer, and there can be no doubt that this force is a function of the absolute temperature and of the concentration of the solution. It is just as evident that the manner of expressing this dependence on the temperature and concentration is important, if a perfect harmony with the gas law is to be expected. It remains to be proven that the simple bombardment of the solute particles will explain the pressure in question in the same way as can be done for a confined gas (*cf.* **28,** pp. 127–131).

VAN'T HOFF was interested mainly in the mathematical side of the relations and not so much in the mechanism or the visualization of the molecular activities. He did not mean to prove that the solute was active and the solvent passive or serving as a mere medium in which the solute molecules might move. This seems clear from his statement: "Before entering on the

proof, which deals with osmotic pressure and semipermeable membranes, *i.e.*, membranes that only allow the solvent to pass, it may be remarked that any notion one may form as to the mechanism producing the osmotic pressure, or the action of semipermeable membranes, is without influence on the reasoning. Thus the question whether the pressure is produced by the solvent or by the dissolved body can be left out of consideration; so too, whether it is dependent on collision or on attractive forces. The action of the membrane too, whether it is as a sieve or by means of absorption, is indifferent. All this is the case because the proof to be given is based on thermodynamics, and is consequently free from assumptions on the mechanism." (See **21**, p. 32.)

As the above quotation suggests, some place the cause of the osmotic pressure in the membrane and others in the combined or independent activities of the solvent and the solute.

The kinetic theory supposes that the pressure is the result of the bombardment of the membrane by the solute, or the difference in the forces resulting in the bombardment by the solute and solvent. An experiment by RAMSAY (**38**) is often quoted in current literature to give support to this view: A palladium bulb filled with nitrogen at 280° C. was surrounded by a stream of hydrogen. The pressure in the bulb increased almost as much as the total pressure of the hydrogen. For all that, serious difficulties present themselves when an attempt is made to make the kinetic theory of gases entirely analogous to the kinetic theory of osmotic pressure.

The hydrostatic theory considers the solvent as playing the chief rôle in the activities, and attributes the resulting pressure to the entrance of the water into the confined solution. The cause of the entrance is supposed to be the attraction which the solute has for the solvent. This attraction might result from the differences in the surface tension of the solvent and the solution (**41, 42, 30**).

According to PICKERING (**36, 37**) and ARMSTRONG (**1, 2**) the osmotic pressure results from the hydrodynamic conditions, *i.e.*, the hydraulic pressure exercised by the extra molecules of hydrone attracted into it by the complexes formed by the solute and hydrone.

Some authors do not express the ideas of VAN'T HOFF correctly in connection with his formula (*e.g.*, HENDERSON (**19**, p. 76)), and others have not observed the limitations which he makes for his deductions concerning the osmotic pressures of dilute solutions (in which neither the aggregate volume of the solute molecules, nor their mutual attraction are of moment (**20**, p. 479). When more concentrated solutions are dealt with, it becomes necessary to modify the simple equation for osmotic pressure for ideal solutions, in a manner analogous to the modification of the gas law by VAN DER WAALS.

MORSE (**31,** p. 99) thinks it doubtful if any proposed general equation for osmotic pressure (although containing suitable terms for all the factors which must be taken into account) would be of any present utility in the case of aqueous solutions, since the value of at least some of these must still be determined experimentally for every solute, at every temperature, and in each individual concentration.

Other more complicated equations have been suggested by various authors, but they are unsatisfactory because of the complexity of forms that make it difficult to ascribe any definite meaning to their empirical constants. (*Cf.* **6, 10, 11, 12, 13, 25, 26, 34, 62.**) MORSE held that if a suitable equation is eventually established it will be the fruit of extensive and painstaking experimental research.

From all this it is clear that if any deductions about the osmotic quantities in plant tissues are to be made from the isotonic conditions of the cell sap and a chosen plasmolyzing medium, the medium chosen should be one whose osmotic pressures have been determined experimentally for different concentrations, at various practical temperatures.

MORSE (**31,** p. 184) established the osmotic pressures for cane sugar between 0° and 80° C., the concentrations varying from 0.1 to 1.0 m. BERKELEY and HARTLEY established a reliable table of osmotic pressures for the same medium, the concentrations going beyond 2 mols. (See **5,** p. 486, and **7,** p. 271.)

For reasons which he explains, MORSE (**31,** p. 97) preferred to work with the weight normal system (gram-molecular weight of solute per 1000 grams of solvent) rather than in the volume normal system (gram-molecular weight of solute per 1000 cubic centimeters of solution). The weight normal system however, is not practical for the investigation of the osmotic relations in plants, so that it became necessary to translate the values of the osmotic-pressure equavalents for various concentrations of sugar solutions, from the weight normal system to the volume normal system. This work was done by URSPRUNG and BLUM. (See **48,** and **29,** p. 442.)

The osmotic value

Since the cell sap is a solution of varying concentration, the term osmotic pressure can be employed in the sense defined above when referring to its various concentrations. The pressure which would have to be mechanically impressed to cause water to be exuded, plus the pressure of the cell wall upon the cell sap, would measure the osmotic pressure. Care must be taken not to confound the osmotic pressure of the sap with the actual pressure of the wall upon the cell sap, or its equilibrant, the turgor. This error occurs rather frequently in current literature. (For examples refer to **53,** p. 209.)

MORSE (**31,** p. 99) pointed out that the osmotic pressure of a given solution can be known only when accurate measurements have been made at different concentrations and at different temperatures with a satisfactory osmometer. It is impossible to do this for a cell or a tissue. In lieu of absolute values relative values may serve the purpose.

DE VRIES (**60, 61**) first attempted to establish such relative values for the osmotic pressure of the cells. He employed the mature epidermal cells of *Curcuma ribicaulis, Tradescantia discolor* and *Begonia manicuta.* These cells had colored cell sap, which helped considerably in noting the first stages of plasmolysis. He reasoned that at this point the concentration of the cell sap must be equivalent to the concentration of the plasmolyzing agent. It would not have been legitimate to conclude that the osmotic pressure (as above defined) of the cell sap in the normal condition was the same as the osmotic pressure of the solution producing incipient plasmolysis. DE VRIES was mainly interested in comparing the relative osmotic pressure of the plasmolyzing agents, so that his procedure would have been correct for his purpose, provided the cells had responded in the same manner towards all of the plasmolyzing agents; later investigations proved, however, that such was not the case.

Since there can be doubt about the relative pressure in atmospheres it is more secure, and just as logical, to speak in terms of the relative concentration of the agent employed, and then draw whatever conclusion is legitimate from the data obtained. URSPRUNG and BLUM (**53**) proposed the term *Osmotic Value,* and expressed it in terms of the molal concentration of the plasmolyzing agent. They recognized that the osmotic value varies with conditions, and distinguished three different osmotic values for the cell: (a) The osmotic value at incipient plasmolysis, symbolized as O_g (g for Grenzplasmolyse); (b) the osmotic value of the cell sap in the normal cell, symbolized as O_n; and (c) the osmotic value of the cell sap when the cell is saturated with water, symbolized as O_s.

Some authors seemed to have noticed the difficulty of expressing the osmotic pressure of the cell sap in atmospheres, and consequently expressed the relative concentration of the agent, calling this the osmotic pressure. (See **53.**) This was not logical and caused a great deal of confusion. Pressure is the force per unit area [$ML^{-1}T^{-2}$] and the concentration is the mass per unit volume [ML^{-3}]. It is wrong to cofound the quantities in this way.

While making a study of the relative merits of different plasmolytic agents, the writer found it advantageous to append the formula for the agent to the symbol O_g. Thus $O_g(KNO_3)$ represents the osmotic value of the cell sap at incipient plasmolysis, when the agent potassium nitrate is employed, and $O_g(C_{12}H_{22}O_{11})$ represents the osmotic value of the cell sap

at incipient plasmolysis, when cane sugar is employed as the agent. Brevity and exactness of expression are of the utmost importance in this field of work (**4**).

Isosmotic and osmotic coefficients

In an *a priori* way it might be supposed that equal molal concentrations of all substances should have equal osmotic pressure; *e.g.*, 101 gm. of potassium nitrate should have the same osmotic pressure as 342 gm. of cane sugar when contained in 1000 cc. of solution.

De Vries (**60**) showed that this is not true, but that the potassium nitrate is about 1.5 times as active osmotically as cane sugar. In general he divided the substances into groups according to their osmotic activity, based upon the value of cane sugar. Giving the sugar the value 1, the series expressing the relative osmotic pressures was 1, 1.5, 2, and 2.5. In order to obtain a series of whole numbers he arbitrarily chose the value 2 for cane sugar, and obtained the series 2, 3, 4, and 5. These numbers were called isosmotic coefficients. If, for instance, the osmotic pressure of a potassium nitrate solution were to be deduced from an equimolar sugar solution, the osmotic pressure of the sugar solution would have to be multiplied by 3/2, this being the ratio of their isosmotic coefficients.

The dissociation of certain substances upon dissolving, is the chief cause for their higher osmotic effectiveness, and for this reason the isosmotic coefficients roughly represent the degrees of dissociation of the salts.

Fitting (**16, 17**) tested out the isosmotic coefficients with greater exactness than De Vries, and found that for specific substances the variation from the values 2, 3, 4, and 5 was considerable; consequently there was no good reason for retaining the basic number 2. He preferred to express the coefficients on the basis of 1 for cane sugar, and called them osmotic coefficients. They are symbolized by i. In effect then, the osmotic coefficient of a given substance is the ratio of the osmotic value of a given cell sap at incipient plasmolysis using cane sugar as an agent, to the osmotic value of the same cell sap using the substance in question as an agent. It follows, for instance, that

$$i = \frac{O_s(C_{12}H_{22}O_{11})}{O_s(KNO_3)} \qquad (3)$$

expresses the osmotic coefficient for potassium nitrate (**4**, p. 24).

The value 1.69 was the best that Fitting could obtain for the potassium nitrate coefficient, using the low concentrations that plasmolyze the epidermal cells of *Rhoeo discolor*. When he determined i cryoscopically he found the value to be 1.78.

The value of i as determined physiologically for potassium nitrate, is invariably lower than the value which is determined by physico-chemical

methods. This is chiefly due to the penetration of the potassium nitrate into the cell. (See **17**, and **4**, pp. 48, 50, 54, 55, 58 and 63.)

When cells of higher O_g values are employed, the i values are considerably lower than that found by FITTING. Reasons for this behavior were given elsewhere (**4**, p. 28).

Certain agents may stimulate the activity of the enzymes in such a way as to increase the O_g values, thus causing considerable variation in i from the ideal value. The guard cells are particularly to be suspected for this possible source of error (**4**, pp. 50 and 65).

FITTING showed that the value obtained for i in the case of certain substances by the plasmolytic method was not constant, when different kinds of plants were used. Employing *Begonia* leaves he obtained the value 1.0 for glycerol, but when he employed the leaves of *Rhoeo* the value was 0.77. Since glycerol penetrates in many cases, this was at first thought to be a sufficient explanation of the discrepancy, but a closer analysis proved that the basic cause still remains to be found.

Constancy of O_g

When the solute can penetrate into the cell-sap cavity during the immersion of the cell in a given medium, the O_g value may increase with the time of immersion. In such a case the medium must be avoided, or at least the most favorable time of immersion must be determined and employed. (See **4**, pp. 22, 56 and 58.) Cane sugar seems to be the most reliable agent for most cells in this respect. (*Cf.* pp. 53, 56 and 62.)

It might be supposed that the true O_g value of a mature cell should not change, *i.e.*, while the normal cell may take in water or transpire water under the influence of various external factors, the nature and quantity of solute might be supposed to remain constant, and therefore the osmotic value at incipient plasmolysis to remain constant also, since the amount and nature of the solute determine the O_g.

It is however not always true that O_g remains constant. The guard cells seem to vary most in their O_g values, when subjected to the influence of various factors. ILJIN (**24**) noted a tremendous increase in O_g values when the plants were placed in water-vapor saturated atmosphere. He used potassium nitrate as an agent, hence an error in measurements might have been suspected. His results were confirmed by STEINBERGER who also used potassium nitrate as an agent. WIGGANS (**63**) also noted an increase using $CaCl_2$ as an agent but it was considerably less than that recorded by ILJIN. The results of URSPRUNG and BLUM (**57**, p. 17) also confirmed the increase in O_g. They used cane-sugar. In a considerable number of experiments which have not yet been published, the writer noted that not all plants show an increase of O_g in the guard cells, when treated in the manner indicated

by ILJIN. Many plants show regular diurnal variation of the O_g of the guard cells.

When a cell can change its O_g value under normal conditions, it must be ascribed to the variation in the nature or the quantity of the solutes in the cell sap or to both. It follows that SCHWENDENER'S theory as to the cause of the opening and closing of stomata must be revised. It is not surprising that the O_g of different tissues in the same plant, or even the same organ of a given plant should be different. While the turgor may be the same, the concentration of the cell sap at incipient plasmolysis can be different, since the quantity of water given off by the cell—until the tension in the wall is relieved—need not be the same in all; nor is the capacity of the cells generally the same. From this it follows that the value O_g does not determine the turgor as some writers (**53**, p. 209) erroneously supposed.

According to the ideas of some investigators, a more or less regular increment of O_g might be expected in the tissues of a plant, starting from below and going upward. URSPRUNG and BLUM found that this view is unfounded. In table III are given some of the results they obtained on the stem of *Urtica* (*cf.* **44**).

TABLE III

VALUE OF O_g (KNO_3) IN THE TISSUES OF THE STEM OF *Urtica*

REGION	PITH	HADROME	INNER CORTEX	OUTER CORTEX	EPIDERMIS
Top	0.35	0.47	0.38	0.44	0.39
Middle	0.4	0.62	0.52	0.55	0.43
Base	0.43	0.66	0.53	0.53	0.59

It is interesting to note that the gradient is in the reverse direction, except in the outer cortex where the value 0.55 is discordant. Furthermore it is to be noted that there is no gradient radially but that the value of O_g seems to be characteristic for a given type of tissue, *e.g.*, the outer and inner cortex have almost the same value in each region of the stem.

Since it is not the gradient of O_g which decides the direction in which the water will flow, there is nothing paradoxical in this result; since the walls of the younger cells offer less resistance than the older ones, young cells will take in more water than the older ones before a condition of equilibrium is established. There are several other variables beside O_g that must be taken into consideration before the actual pressure existing in the normal cell can be calculated.

According to HANNIG (**19**) hydrophytes manifest a greater O_g in the leaves than in the roots.

URSPRUNG and BLUM determined that the O_g in the root tips is greater near the tips and decreases regularly in the first 8 mm. from the tip.

The cells of a given tissue do not as a rule have the same O_g value (**4**, p. 56); nevertheless the mean value is, as a rule, characteristic for that tissue. This was shown nicely in a study made by the writer on herbaceous and woody plants. All of these plants grew in the same well-cultivated garden soil. The results are recorded in table IV.

As investigations, the results of which have not as yet been published, proved that some tissues show a considerable diurnal and annual variation in their O_g value, the tests were made at about the same time of the day, and about the same time of the year. An effort was made to have the conditions for each woody plant the same as for a given herbaceous plant. The measurements were made in the early part of the day because the effect of the sun on the O_g of the guard cells is quite different in different plants.

The difference in the mean values for the epidermis and the guard cells in the herbaceous plants is 0.0875 in favor of the guard cells. This is very little. In woody plants it is also negligible, *i.e.*, 0.075. The difference between the values for cells of the epidermis and spongy parenchyma (chosen to represent the assimilating tissue) in the herbaceous plants is 0.2325, which is considerable. So also is the difference considerable between the similar tissues in the woody plants, *i.e.*, 0.261.

Comparing the values, tissue for tissue, it is seen that the cells of woody plants have considerably higher values than those of the herbaceous plants. There is not a single exception, when the data are compared horizontally across the table. The mean value for the epidermis in the woody plants is greater than that for the same tissue in the herbaceous by 0.2025. The mean values for the guard cells differ by 0.19 in favor of the woody plants. In the same order the difference in the mean values for the spongy parenchyma is 0.23. These differences are significant, since, other things being equal, the cells with less elastic walls demand a higher concentration in the sap to draw the same amount of water. It is furthermore significant that the difference 0.225, 0.19 and 0.23 are almost equal.

The osmotic value of a given cell or tissue can vary under the influence of various factors (**3**). Decreasing temperature causes an increase in O_g, and so also does an increase which is sufficient to injure the protoplasm (**3**, p. 54). According to BÄCHER, light seems to increase the O_g value, but the results of investigations by the writer, which will be published later have shown that different tissues need not respond in the same way in this respect. Red light is more effective than green, and blue less effective in increasing the value. The O_g increases also with drought and with decreasing relative humidity of the atmosphere.

TABLE IV

COMPARATIVE O_g ($C_{12}H_{22}O_{11}$) OF THE TISSUES IN LEAVES

HERBACEOUS PLANTS						WOODY PLANTS					
DATE 1925	TIME OF DAY	PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PARENCHYMA	DATE 1925	TIME OF DAY	PLANT	LOWER EPIDERMIS	GUARD CELLS	SPONGY PARENCHYMA
August 17	8:30 A.M.	*Solanum nigrum*	0.425	0.675	0.575	August 14	8:00 A.M.	*Ligustrum vulgare*	0.65	0.75	0.975
August 17	6:30 A.M.	*Crambe maritima*	0.375	0.65	0.65	August 14	6:30 A.M.	*Hibiscus syriacus*	0.6	0.55	0.8
August 17	7:00 A.M.	*Hesperis matronalis*	0.55	0.6	0.725	August 22	6:30 A.M.	*Fraxinus excelsior*	0.775	0.825	1.1
August 18	6:30 A.M.	*Euphorbia lathyrus*	0.25	0.275	0.575	August 21	6:30 A.M.	*Cytisus laburnum*	0.675	0.9	1.075
August 18	7:00 A.M.	*Osmunda regalis*	0.475	0.425	0.875	August 22	7:00 A.M.	*Robinia pseudacacia*	0.525	0.7	0.9
August 18	8:00 A.M.	*Polygonum orientale*	0.375	0.55	0.675	August 21	7:00 A.M.	*Liriodendron tulipifera*	0.6	0.675	0.825
August 27	9:00 A.M.	*Musa sinensis*	0.4	0.35	0.45	August 21	8:00 A.M.	*Hedera helix*	0.55	0.575	0.675
August 29	7:30 A.M.	*Datura stramonium*	0.3	0.425	0.55	August 29	7:30 A.M.	*Crataegus oxyacantha*	0.55	0.575	0.85
September 4	7:30 A.M.	*Acanthus spinosus*	0.475	0.525	0.625	September 4	7:30 A.M.	*Castanea sativa*	0.575	0.625	0.775
September 5	8:30 A.M.	*Dioscorea batatas*	0.35	0.375	0.6	September 5	8:30 A.M.	*Populus nigra*	0.5	0.575	0.625
		Mean values	0.3975	0.485	0.63			Mean values	0.6	0.675	0.861

According to MEIER (**27**) the O_g is greater for plants growing in rocky and mountainous regions than for plants growing in the plain.

These examples prove that the nature and quantity of solute in the mature living cell, need not be constant, and that variations in this respect are seemingly to be considered as adaptive responses to the plant's environmental conditions.

Suction tension

The cells, as they occur in the plant, can obtain the necessary water directly from a given source, or from another cell, by virtue of the force which tends to bring the solute and water together. We are not interested here in the nature of this force. It will suffice for our purpose, that such a force exists and that we can express the force numerically per unit area. By experiment it has been shown, that this force which tends to draw or press water into the cell, can be counteracted entirely or in part, by the pressure of the wall against the sap. The influx of water tends to dilute the sap and increase the pressure of the wall; consequently a point is reached at which the influx of water must cease, even though the sap is still fairly concentrated. Just at what instant this point is reached depends on many factors and cannot be decided by the osmotic value alone as many have erroneously supposed.

The cell may be regarded as an entity under tension, capable of absorbing water in proportion to its tension. If the tension is real or not need not be decided for the moment. Just as osmotic pressure is an ideal pressure, which is not expressed directly and mechanically, so also is this tension in the cell an ideal quantity; furthermore, we need not necessarily commit ourselves to the view that water is drawn into the cell rather than forced into it, by using the term, "suction tension of the cell," any more than the acceptation of the expression "osmotic pressure," as above defined, demands that we definitely adhere to the kinetic theory of osmosis. These terms refer to the nature of the experimental data obtained, and not necessarily to the nature of the forces underlying the phenomena.

There are other cases in physics in which quantities are known to exist, even though they do not receive expression in such a manner that we can directly take cognizance of their dimensions, notably the cases involving potential energy. The potential energy is expressed in units having the dimensions of extension, mass, and acceleration; but the mass does not move through a given distance, and there is no acceleration of the mass.

URSPRUNG calls this tension "suction force" and distinguishes between the "suction force of the contents" of the cell and the "suction force of the cell." The term is not well chosen, since it is not meant to express the total force which the sap or the cell exerts, but the force per unit area.

This is the sense in which URSPRUNG and BLUM constantly use the term and they express the quantity in atmospheres and not in the unit of force. It is unfortunate that URSPRUNG and BLUM (**53**) did not note the error at the time when they so ably began to clear up the confusion which was caused by the misuse of the term "osmotic pressure."

The term "suction force" does not satisfy the dimensions of the quantity which it is intended to express. The dimensional equation of force is:

$$[F] = [MLT^{-2}]$$

and that of pressure or tension is:

$$[P] = [ML^{-1}T^{-2}]$$

URSPRUNG and BLUM (**53**, p. 202) formulated the law of the "suction force" as follows: "Es ist Saugkraft der Zelle = Saugkraft des Inhaltes – Wanddruck."

The suction tension of the contents of the cell is not identical with the osmotic value of the contents of the cell, but only equivalent. The suction tension of the contents of the cell is given by the equivalent in atmospheres of the molal expression of the osmotic value of the same, which may be symbolized:

$$S_i \backsimeq O_m \qquad (4)$$

The influx of the water causes tension in the wall which acts antagonistically to the suction tension of the contents; hence the difference between the numerical value of the suction tension and the numerical value of the wall pressure is the resultant suction tension of the cell as an entity. The authors have symbolized their law by:

$$S_z = S_i - W \qquad (5)$$

in which S_i is the "suction force" (suction tension) of the contents of the cell, W the wall pressure, and S_z the "suction force" (suction tension) of the cell.

This equation brings the error of calling the suction tension "suction force" into relief, since the minuend and the subtrahend must be expressed in the same units, in order that the operation may be logical. Suction tension may be considered a negative pressure, and can be expressed in atmospheres. According to the equation, however, units of force (*e.g.*, pounds) should be subtracted from units of pressure or tension (*e.g.*, atmospheres), and the result should be expressed in units of force, according to the terms, but the authors actually express the results in atmospheres; all of which is quite illogical.

From equation (5), it is evident that if the wall pressure is constant, the suction tension of the cell varies in the same degree as the suction tension of the contents of the cell; *e.g.*, two cells having the same wall pressure at a given instant have the same suction tension when the suction tension of the contents is the same in both cells. With the influx of water, however, the suction tensions of the two cells will probably soon differ, since the capacities of the cells and the coefficients of elasticity of the walls will probably not be the same.

In general the suction tension of the contents decreases with the influx of water, the wall pressure increases, and consequently the suction tension of the cell decreases.

The equation shows clearly that at incipient plasmolysis, the suction tension of the cell is equal to the suction tension of the contents of the cell, since the wall is completely relaxed and its pressure is zero. Cases are on record where the loss of water in certain cells of specialized tissues was so great that the walls were not merely completely relaxed but even folded like a spring, so that they could exert a negative pressure; consequently the suction tension of the cell was greater than the suction tension of the contents of the cell. It is furthermore clear from the equation (5) that when the wall pressure equals the suction tension of the contents of the cell the suction tension of the cell is zero, *i.e.*, the influx of water must cease. The use of this equation presupposes that the cell is excised, and that the source from which the cell is to receive water is pure water. In a series of cells, in which these relative values form a regular gradient, it is reasonable to suppose that the water will flow from cell to cell in the direction of the gradient, but it would be difficult, if not impossible, to express the absolute suction tension which actually exists between any two of the cells as they lie normally in the plant.

HÖFLER (**22**, p. 290) first tried to show graphically the relation of three quantities expressed in the equation for the various stages, as the cell passes from a condition of incipient plasmolysis to the condition of saturation. His graphs are given in fig. 1. The wall pressure variations are given by the straight line (G O_T). URSPRUNG assumed that the tension in the wall increased directly as the volume increased, so that the straight line is determined if the two points G (volume at incipient plasmolysis, with wall pressure zero), for incipient plasmolysis, and O_T (volume determined and pressure determined), for the point of saturation, are known. The curve O, O_t and O_T is given by the variation of the suction tension of the contents of the cell (determined from the respective osmotic values), while passing from the condition of incipient plasmolysis to the point of saturation. The suction tension of the cell is given by the vertical lines (S, S) between the curve and the straight line.

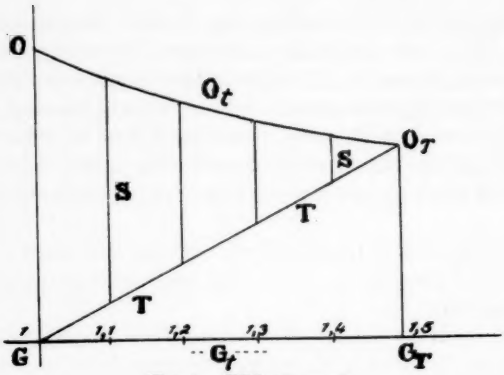

FIG. 1. Höfler's graph.

In fig. 2 are given the relations of the various quantities as they were found in the illustrative example which follows. It is to be noted that the axis of ordinates has been carried to the left of the point where the volume is unity (as given by HÖFLER) in order to avoid confusion. The straight line has been carried beyond the abscissa, to suggest that a cell, in which the cell wall becomes folded, can develop a suction tension greater than the suction tension of the contents of the cell.

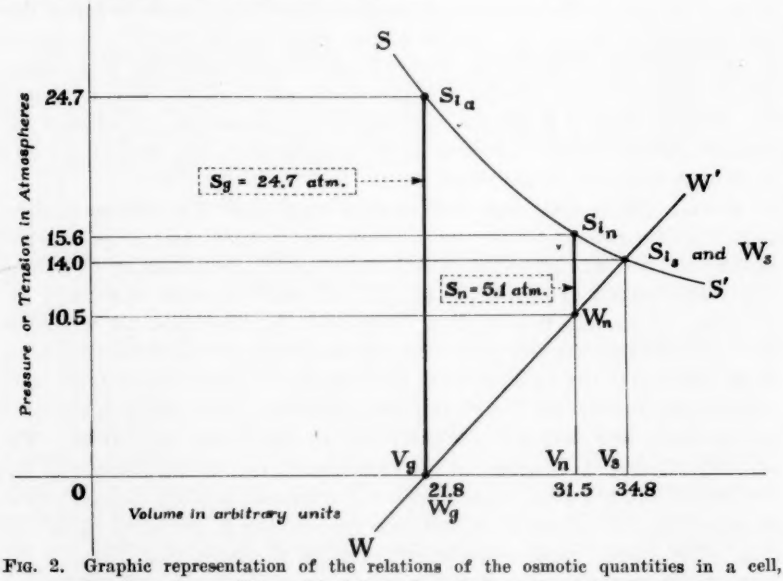

FIG. 2. Graphic representation of the relations of the osmotic quantities in a cell, passing from the condition of incipient plasmolysis to the state of saturation.

The volume of a cell in the normal state was found to be 31.509 (arbitrary units). At incipient plasmolysis the same cell had a volume 21.799, and at the point of saturation 34.779 units. These values were laid off to scale along the abscissa. The osmotic value of the cell sap at incipient plasmolysis [$O_g(C_{12}H_{22}O_{11})$] was found to be 0.78 mol, which is equivalent to a suction tension of the contents of the cell (S_{i_g}) of 24.7 atmospheres (obtained from the tables of URSPRUNG and BLUM according to the determinations of MORSE).

The osmotic value of the contents of the cell at the point of saturation (O_s) was obtained from O_g as follows: Since the concentration varies inversely as the volume (in the volume normal system), when water is added to a given solution, we may write:

$$\frac{O_s}{O_g} = \frac{V_g}{V_s} \text{ and } O_s = O_g \cdot \frac{V_g}{V_s} \tag{6}$$

Replacing the values O_g, V_g and V_s by 0.78 mol, 21.799 units and 34.779 units respectively, in equation (6), the value 0.49 mol was obtained for O_s. This is equivalent to 13.987 atmospheres (S_{i_s}).

In a similar manner the osmotic value of the contents of the cell in the normal condition was obtained. It was 0.54 mol (O_n) which is equivalent to 15.6 atmospheres (S_{i_n}).

The values for S_i were then plotted along the ordinate against the units of volume, and the three points of the curve were determined. The straight line was determined by the two points V_g (21.8, 0) and S_{i_s} coincident with W_s (34.779, 13.987).

The vertical lines were then dropped from the three known points of the curve for the variation in S_i (S, S′). The vertical for S_{i_n} intersected the straight line representing the variation in volume (W, W′) at the point W_n.

The vertical distances between the curve and the straight line were drawn heavier in order to emphasize the fact that these vertical differences represent the suction tensions of the cell in the different stages. At incipient plasmolysis (S_g) the suction tension was 24.7 atmospheres, in the normal condition (S_n) it was 5.1 atmospheres. The vertical distance from V_n to W_n (representing the wall pressure existing in the normal cell) was 10.5 atmospheres.

The wall pressure of the normal cell could also have been obtained by direct calculation. As was mentioned previously, URSPRUNG assumes that the wall pressure varies directly with the increment in the volume of the cell; therefore we may write:

$$\frac{W_n}{W_s} = \frac{V_n - V_g}{V_s - V_g}, \text{ and } W_n = \frac{V_n - V_g}{V_s - V_g} \cdot W_s \tag{7}$$

When W_n and W_s are the wall pressures, respectively, at normal and saturation points $(V_n - V_g)$ and $(V_s - V_g)$ are the respective increments in the volume. By inserting the known values in equation (7) the value of W_n was found to be 10.5 atmospheres.

Another method of finding the suction tension of cells, in which no account is taken of related osmotic quantities, is based on the principle that a cell which is in dynamic equilibrium with the solution in which it is immersed (will neither increase nor decrease its volume) has a suction tension which is numerically equal to the osmotic pressure of that solution. In practice, that concentration of the given agent in which the immersed cell does not vary its volume must be determined.

URSPRUNG and BLUM have employed this method and compared it with the first and found the results satisfactory. It is simple and yet reliable.

These two methods are particularly adapted to the investigation of the relative values of the suction tensions of the cells in a given tissue, organ, or entire plant.

The associated authors made a painstaking analysis of the suction tensions in the various regions of a plant, under varying conditions. The results were very gratifying and they warrant the expectation that new vistas in plant physiology have been opened, since hitherto unsolved problems have yielded to their methods.

It is evident from the general trend of the literature that a steady gradient of the osmotic value at incipient plasmolysis was thought to exist in the direction of the streaming water (**39, 40, 18, 14**).

To illustrate that the conditions need not be so, the following study of the writer will serve. When the stems of certain succulent plants are cut and left to dry it is noted that the older leaves wilt first and shrivel up, while the younger ones remain turgescent much longer, and in their axes young shoots begin to sprout. PRINGSHEIM claimed that the younger portions had a higher osmotic value at incipient plasmolysis and hence could draw water from the older portions as from a reservoir. To check his claim the stems of *Sedum telephium* were used. The general behavior was as stated above. The result of the mean osmotic values at incipient plasmolysis $[O_g(C_{12}H_{22}O_{11})]$ for different tissues of the older and younger leaves, at different times after the stems were cut, are given in table V. The leaves were numbered beginning at the apex, and proceeding from the younger leaves to the older ones. At each successive test a different leaf was chosen since injury might affect the osmotic value.

It will be noted in the first two tests, which were made immediately after cutting, that the older and younger leaves showed equal values in the respective tissues, except in the lower epidermis, where there was a differ-

TABLE V

$O_g(C_{12}H_{22}O_{11})$ IN OLDER AND YOUNGER LEAVES OF *Sedum telephium*

ORDER OF LEAF	DAYS AFTER CUTTING	$O_g(C_{12}H_{22}O_{11})$ OF THE TISSUES				
		UPPER EPIDERMIS	LOWER EPIDERMIS	GUARD CELLS	PALISADES	SPONGY PARENCHYMA
38	0	0.25	0.225	0.275	0.450	0.450
5	0	0.25	0.200	0.275	0.450	0.450
32	2	0.25	0.225	0.225	0.350	0.350
6	2	0.25	0.250	0.225	0.425	0.350
28	6	0.25	0.225	0.300	0.375	0.375
7	6	0.30	0.275	0.300	0.375	0.375
25	8	0.25	0.225	0.325	0.400	.....
8	8	.....	0.275	0.350	0.375	.....

ence of 0.025 mol in favor of the older leaf. On the second day after cutting, the lower epidermis of the younger leaf had a greater O_g than the equivalent tissue of the older leaf. The difference was 0.025 mol. On the sixth day the younger leaf again showed a higher O_g value in the epidermal tissues but not in the assimilating tissues. On the eighth day the guard cells showed a difference in favor of the younger leaf and again in the lower epidermis, but the palisades showed a higher value in the older leaf.

Evidently the differences in favor of the younger leaves (similar and unsatisfactory results were obtained for the tissues of stems) were never consistently sufficient to support the view of PRINGSHEIM.

If it is recalled that the coefficient of elasticity is less in the young cell walls than in the older ones, and that accordingly the wall pressure (other things equal) is less in the younger cells, it becomes evident that the suction tension in the younger cells is greater; which fact witnesses to the correctness of PRINGSHEIM's basic idea, that the younger tissues obtain their water from the older tissues.

In general, the results of investigations which had as object to prove that the O_g gradient was in the direction of the streaming water, proved to be disappointing. (See **45, 46, 47, 50, 51, 52.**)

On the other hand, a gradient for the suction tensions (S_n) in the direction of the streaming water has been established on several occasions by URSPRUNG, BLUM and HYOZ. From the tips of the roots to the transpiring parenchymatous cells of the leaves, URSPRUNG and BLUM established a regular gradient for S_n, while a similar gradient did not exist in the same plant for O_g. HYOZ (**23**) established the S_n gradient in the leaves of *Hedera helix*. On the cross-section of the stem, URSPRUNG and BLUM found the S_n gradient to run from the water conducting hadrom to the epidermis. In the roots

the conditions were reversed up to the endodermis; here an unexpected and sudden drop was found. They speak of this as the "endodermis jump" (**54, 43, 44**).

This jump is difficult to explain. URSPRUNG and BLUM claim to have proven that a "polar differentiation" exists in the endodermis cells and again in the vascular parenchyma. The accepted S_n value is supposed to be the mean value of a suction tension on one side of the cell, which is considerably greater than the mean, and a suction tension on the other side of the cell which is considerably less than the mean (**58, 44**). This is illustrated in the table in which the values obtained by them on the root of *Vicia faba* are given.

TABLE VI

S_n OF THE TISSUES OF THE ROOT OF *Vicia faba*

TISSUE		S_n	S_n
5th cortical layer			3.2 atm.
6th cortical layer			4.0 atm.
Endodermis	outer side	4.7 atm.	2.6 atm.
	inner side	0.5 atm.	
Pericycle			1.6 atm.
Vascular parenchyma	outer side	4.5 atm.	2.6 atm.
	inner side	0.7 atm.	

URSPRUNG (**58, 44**) outlines his method of measuring the polar differentiation of the suction tension and gives most interesting data, but does not explain how such a strange physical condition, which, apparently at least, contradicts the law of equilibrium, can exist continuously in certain cells. Since the "suction force" law should apply in the particular case, the cause must be sought either in a difference of the molal concentration of the contents of the cell, or in a difference in the tension of the cell wall. It is difficult to see how a considerable difference in the concentration of the contents of the cell should continue to exist for a great length of time (perhaps constantly) and then in a gradient opposite in direction to the influx of the water. It is even more difficult to see how a difference of tension in the cell wall could continue to exist in different parts of the same wall. Even if such a difference could be proven, the final effect of all the different pressures upon the contents of cells should be the mean value of all of the different pressures, and the mean pressure should be equally conducted in all directions within the contents of the cell; thus it is difficult to see how the suction tension of a cell should be different at any two points on a cell.

The method employed admits of the possibility that one and the same cell might manifest different suction tensions at different times (since the

measurements are made successively and not simultaneously and each measurement demands a considerable amount of time). This condition however is not probable, since the lower values were invariably found on the same relative side of the cell.

The measurement of polar differentiation in suction tension is complicated and difficult, but the clearing up of this mystery seems important enough to warrant patient and intense investigation.

If due allowance is made for the faulty nomenclature of the time, it is easy to see that FITTING (**15**) had the correct impression of the osmotic relations in the cell and the real cause of the influx of water into it, when he studied the relations in desert plants in 1911. He interpreted the reason for the high concentration of the cell sap and the low tension in the wall, and said that the "suction force" (Saugkraft) must be very high.

It is interesting to note that in hydatophytes just the contrary condition prevails, *i.e.*, the suction tension is very low, the concentration of the sap being low and the tension in the wall being high.

Mesophytes and hygrophytes take intermediate positions between these two extreme cases (see **29**, p. 500).

Another method for measuring the suction tension which URSPRUNG and BLUM (**56**) devised is particularly well adapted for measuring the mean S_n of tissues, organs or entire plant sections. It is called the simplified method. Whole sections are immersed in the agent, and the concentration at which the solution and section are dynamically balanced is determined. The osmotic pressure of that particular solution is taken to be numerically equal to the mean S_n of the section.

With the aid of this method URSPRUNG and BLUM determined the periodic variations of the suction tensions in response to external factors. It was found that the moisture of the soil exercises the greatest influence on the S_n. The variations depend on the relative resistance which the plant encounters in extracting moisture from the soil rather than on the absolute degree of dryness. This fact suggests (**55**) a practical method of measuring the relative resistances of different kinds of soils to the extraction of water by plants. The relative humidity was found to be the next factor in importance regarding the influence exercised on the suction tensions of the plant.

As might be expected from the effect of the external factors, environment exercises a great influence on the suction tension, which in turn affects the general character of the plant. The suction tension of an organ in a plant remaining in the same location, can vary from 10 to 20 atmospheres in a relatively short time. Different species growing in the same place can manifest widely different suction tensions simultaneously. To judge the

effect of environment, it is best to obtain simultaneous measurements on the same species in different localities. This is not as difficult as it appears to be, since the suction tension of cells and organs remains constant for a long time after being excised, if they are preserved in paraffin oil (**23**). BLUM (**8, 9**) made many comparable measurements of suction tension of different species in different localities, and used the values of simultaneous measurements made on *Taraxacum* and *Bellis* growing in those localities as standards. The relation between the suction tension and alpine habitats is illustrated in table VII. The principle that the suction tension increases with the dryness of the soil was demonstrated in the plains as well as in the mountains. The differences in suction tensions are undoubtedly due to the differences in the water balance, which is expressed by the ratio of the transpiration to the absorption; it is symbolized:

$$B = \frac{T}{A} \qquad (8)$$

in which T expresses the transpiration and the A the absorption. (See **59**.)

TABLE VII

SUCTION TENSION OF *Lotus corniculatus* IN ALPINE HABITATS

HABITAT	S_a IN ATMOSPHERES
Moist sod	9.5
Alpine meadow	14.5
Rock crevice	21.5
Rock fissure	26.5
Humus belt	29.5
Rubble	34.5

MOLZ (**29**) made an extensive and valuable study of the relation of suction tensions and environments. His findings corroborate the views of URSPRUNG.

Summary

Regarding the nature of osmotic pressure, widely divergent views have been expressed. One theory places the cause of the phenomenon in the greater bombardment of the membrane by the solute particles than by the solvent particles. The other prominent theory considers the solvent as playing the chief rôle, and is known as the hydrostatic theory.

The use of the term "osmotic pressure" does not imply that the kinetic theory must necessarily be adhered to.

Osmotic pressure is defined as the difference of pressure on solution and solvent, which produces a condition of equilibrium, such that there is no tendency of the solvent to flow in either direction.

VAN'T HOFF inclined to the kinetic theory but did not commit himself. He showed the similarity between the mathematical expression of the gas law and the mathematical expression of the osmotic law. He found the constant to be about the same for both. He used the rather inexact data of PFEFFER as basis for the proof.

The osmotic value of a given cell sap is the molal concentration of an agent which is in dynamic equilibrium with the cell sap. The osmotic value at incipient plasmolysis is symbolized by O_g, at normal by O_n, and at saturation by O_s. It is convenient to append the formula for the agent employed, *e.g.*, $O_g(C_{12}H_{22}O_{11})$.

For each osmotic value there is an equivalent suction tension of the cell sap. The suction tensions for the different stages are symbolized respectively, S^1_g, S^1_n, and S^1_s.

The isosmotic coefficient of a salt is the number in the series established by DE VRIES, which expresses roughly the osmotic activity of the substance, and the degree of dissociation.

The osmotic coefficient of a salt is the ratio of the osmotic value of a cell sap, in terms of cane sugar, to the osmotic value of the same, in terms of the given salt. It is symbolized by i, *e.g.*,

$$i = \frac{O_g(C_{12}H_{22}O_{11})}{O_g(KNO_3)}.$$

The osmotic value of a cell may change under the influence of external factors.

The osmotic value of neighboring cells need not be the same, even though they belong to the same tissue. The average value for a given tissue is characteristic for that tissue.

No regular gradient of O_g could be established in the direction of the streaming of the water in the plant.

The suction tension of a cell represents the positive or negative pressure which causes the influx of the water into the cell. The use of the term does not imply that the hydrostatic theory is adhered to. This term should be used instead of URSPRUNG'S "suction force." The numerical value is given by URSPRUNG'S law which states that the "suction force" of a cell equals the suction force of the contents of the cell less the wall pressure. It is symbolized by

$$S_z = S_i - W$$

The relations of these quantities are conveniently represented by graphs first employed by HÖFLER.

There are various methods used for the determination of suction tensions in cells, tissues, or entire plant sections.

There is a suction-tension gradient in the direction of the streaming water in the plant.

The suction tension is influenced by external factors. The moisture of the soil and the relative humidity of the air are the most important.

The suction tension of the root hairs may be used as an indicator for determining the relative resistance which soils offer to the influx of water into the plants.

The suction tension of plants depends upon the habitat.

According to URSPRUNG there can be a polar differentiation of suction tensions in certain cells.

UNIVERSITY OF DAYTON,
DAYTON, OHIO.

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PLANT NUTRITION STUDIES IN RELATION TO THE TRIANGULAR SYSTEM OF WATER CULTURES*

HARRY F. CLEMENTS

(WITH SIX FIGURES)

A review of the literature bearing on the subject of plant growth in culture solution reveals the fact that criteria used in determining the optimum combination of salts have consisted of measurements of the external portions of plants. Length of tops and roots, green and dry weights of tops and roots, amount of water of transpiration, etc., have served as the means of describing the plants grown in the cultures by the various investigators.

In the course of this article, the term "best" plant will be encountered repeatedly. In order to define the term, it is necessary to consider the purpose for which the plant is grown. Obviously, a plant which is considered best for forage purposes will not be the best so far as seed production is concerned, and *vice versa*, but the very fact that some plants are grown for forage and others for seed, implies that they were capable of being developed in the one direction or the other by man. Thus, when a phenomenon, such as the physiological balance of salts for any particular plant, is to be studied it seems only logical that the plant should be considered from the viewpoint of the plant itself and not from that of the animal. Thus, the general term "best" or "normal" plant refers to one which, more specifically, produces sufficient vegetative growth to insure reproduction of the highest order.

With such a plant in mind, it will be well to consider the indices of normal plants which have been mentioned previously. Measurements of roots must yield conflicting results since GARNER and ALLARD (3) have shown that roots often respond with increased vigor to stimuli which suppress top growth. Obviously, roots can not provide for optimum reproduction, and since the tops are concerned with the survival of the species, attempts must be made to obtain measurements from them. Likewise, if roots become a limiting factor, that condition will be manifested in the expression of the tops.

The amount of transpiration water has been used as an indicator. Here, too, it seems certain that many obstacles will be encountered which will tend to confuse rather than to explain results. At best, the amount of water

* Published with the consent of the Experiment Station Director.

which a plant will lose will depend on a great multiplicity of factors, such as the concentration of the culture solution, succulence of the plants, etc. It seems improbable, therefore, that such a criterion could serve the office of determining what constitutes a good plant.

When such measurements as length and weight of tops are taken, again the question arises: "Is the tallest or the heaviest plant necessarily the best one?" Apparently it is not. Thus, any plant which shows a tendency toward succulence will in all probability be taller than one which shows a tendency to be higher in dry material. Green and dry weights would be affected in a similar manner.

It becomes apparent that any attempt to determine a well balanced plant —one that develops its usual functions normally—must be based not only upon quantitative data but must also take into consideration something other than the external expression of the plant.

The possibilities of using measurements of the external features of plants as standard criteria, therefore, seem to be quite thoroughly exhausted. It is probable that criteria based on the combined measurements of the internal and external features will yield more fruitful results. KRAUS and KRAYBILL (**5**) have pointed out that the external appearance of a plant is simply an expression of an internal condition. GARNER and ALLARD (**3**), on the other hand, have demonstrated that the behavior of plants depends to a large degree on the relative length of light exposure. NIGHTINGALE (**8**), however, emphasized the fact that although light exposures of various durations modify the behavior of the plant, they first cause a change in the internal composition of the plant which then is followed by a response in external appearance. In view of these tremendously important generalizations, it seems that by combining measurements of both the external and internal conditions, a criterion of what constitutes a normal plant may be established. Any attempt to demonstrate that one's best plant must be his tallest, or his heaviest, from either a green weight or dry weight basis would probably end in failure as the result of unreliable criteria. True, there are cases in which a plant may be an excellent one and be the tallest of a series, but when groups of workers differently located and with different points of view use the same measurements, only confusion from the reader's point of view can be the result.

No attempt will be made here to present an extended review of the literature on this subject. TOTTINGHAM (**13**) in 1914 gave a thorough review of the literature in his paper, and since that time several hundred papers have appeared dealing with water cultures. Many phases of plant development and behavior have been approached and many valuable and interesting results obtained. Such topics as the effect of the H-ion concentration on growth, the absorption and effect of certain ions or salt combinations,

rate of absorption, and the application of biometry seem to have attracted the greatest share of interest. Many times the data of one man have seemed to be diametrically opposite to those of another who used exactly the same plant and approximately the same conditions of temperature and humidity. Some have supposed that external conditions could not be duplicated and have gone into the work of perfecting control chambers. It seems improbable that any system having such a great range of salt proportions could be disrupted by a few degrees difference in temperature or a few per cent. difference in humidity. If this were the fact, the triangular system would be absolutely useless in our research problems and all that could be obtained with this technique would be to show the uniqueness of the method itself without any illuminating facts concerning the growth and behavior of plants. It does, however, seem that the relative length of day has not been given proper consideration in this type of experimentation.

Thus, an attempt will be made in this paper to throw light on the organic nutrition of the plants grown in the triangle and to study the internal conditions in relation not only to the various combinations of salts, but also to the various exposures of light.

Methods

Seed treatment

The Scotch Beauty field pea was used in this experiment for no other reason than that legumes, with few exceptions, have not been used in this type of experimentation. In order to obtain uniform seeds, the peas were selected according to their weight. Several hundred seeds were weighed separately, the distribution of these weights was obtained and the two central classes were established from the data. Approximately five thousand seeds were then selected ranging in weight from 0.2193 to 0.2445 grams. Before planting, these seeds were treated in a formalin solution (1:250) for thirty minutes, washed thoroughly in distilled water, and allowed to soak in distilled water for ten hours. They were germinated between folds of moist blotting paper in pans which were kept at a temperature ranging from 29° to 31° C. When the roots were approximately 2.5 cm. long the seedlings were transplanted to the culture jars. These jars were of the glazed crock type and had a capacity of twelve liters. Thus it was possible to grow forty seedlings in a single culture. The importance of large numbers of plants in a culture is obvious, and, according to Hibbard and Gershberg (**4**), necessary. Five seedlings were planted into each of eight corks which were fitted into the cover of the crock.

The solutions used were those having an osmotic pressure of one atmosphere used by Shive (**11**). The combinations of salts used were as follows:

$Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4. Iron was added in the form of iron tartrate. It was found that when other iron salts were used they invariably caused a partial precipitation of the other culture salts. Twenty-five cc. of a 0.2 per cent. solution of the iron salt were added to each culture at the time that the solutions were changed. Likewise, an equal quantity of 0.2 per cent. solution of $MnCl_2$ were added to the cultures to overcome a tendency on the part of the plants to be chlorotic.

The twenty-one culture jars were placed in a greenhouse on a rotating table which was kept in continuous operation during the entire experimental period. The solutions were changed once each week, and the experiment was continued for about seven weeks. At the end of each series the following data were obtained: green and dry weights of tops and roots, respectively, length of tops and roots, and various forms of carbohydrates of the tops, ash content of tops and roots, total nitrogen of the tops, and the nitrate content of both roots and tops. As the experiment progressed some of these indices were omitted after it was evident that they would aid little in the interpretation of results. The plants of all three series were free from disease.

Chemical methods

A. Desiccation.—Drying the plants was effected in an oven heated to 90° C. for the first half hour to insure killing all enzymes, after which the temperature was reduced to 65° C., following the recommendations of Tottingham and Link (**6**).

B. Carbohydrates.—Three-gram samples of the dried material were used in the carbohydrate analyses. These samples were subjected to eighteen hours of continuous ether extraction. They were allowed to dry and were then subjected to a two-hour extraction in 90 per cent. ethyl alcohol.

Between the time that series 1 and 2 were run, collateral work with other plants showed that preserving the samples in alcohol instead of drying, considerably reduced the variation one found between two portions of the same sample. Hence, in series 2 and 3, approximately one half of the peas from a single culture was placed in alcohol immediately after length and weight measurements were taken. Such samples were later ground in a plate grinder, more fresh alcohol added, and the whole refluxed for two hours. The rest of the procedure was the same as in those cases where dried material was used.

a. *Simple sugars and sucrose.*—Following the alcohol extraction, the mixture was filtered. The filtrate was evaporated almost to dryness under reduced pressure which was so regulated that the alcohol boiled at never more than 50° C. The residue was taken up in water. Sometimes it was

difficult to get out all of the material and in such cases the water was either warmed or some ether was added. This watery extract was then clarified with Horne's dry lead. Disodium phosphate was used in deleading, following the recommendation of ENGLIS and TSANG (**2**). They find that carbonates, sulphates, and oxalates cause losses of reducing substances while disodium phosphate causes practically no loss. Their findings were also verified in this laboratory. After filtration, the filtrate was diluted to 500 cc.

For the sucrose determinations, aliquots of the above volume were treated according to the chemical method outlined in the Official Methods.

The reducing power of these solutions was obtained by the use of the SHAFFER-HARTMAN (**10**) modification of the MUNSON-WALKER method. Although there seems to be some question in the minds of the Standardization Committee (**1**) of the American Society of Plant Physiologists concerning the use of this method, there appears to be no definite criticism of the method. In this laboratory, the method was compared with the official MUNSON-WALKER method and showed every indication of being the more reliable of the two. Furthermore, in the process of clarification, even after the most severe filtration, there always remained some precipitated lead salt in a fine degree of suspension. After long standing this settles out. If the gravimetric method is used, the solutions must be allowed to stand until this has settled out; otherwise this, too, would be weighed as cuprous oxide. On the other hand, this suspension does not in any way affect the iodometric method. Although the permanganate method is accurate, it entails considerable routine and where many analyses are to be made, time becomes a limiting factor. It was possible in this laboratory for a single person to complete twenty analyses in an hour. It is felt that this method should be given a wider use. So, in spite of the recommendations of the Committee, the SHAFFER-HARTMAN method was used in this laboratory.

b. *Starch.*—The residue from the alcohol extraction was washed off the filter paper with water and the mixture was boiled for five minutes to thoroughly gelatinize the starch; it was then cooled, and 10 cc. of fresh saliva added. The mixture was allowed to digest over night in an incubator heated to 37° C. This digestion is usually complete after a much shorter digestion, but the routine was so arranged that this digestion began at the end of the day and was allowed to continue until the next morning. The mixture was filtered and H_2SO_4 was added to the filtrate in such quantities that the latter represented a 2.5 per cent. solution of the acid. The solution was then heated for 1.5 hours on a boiling water bath. This solution was cooled, neutralized with NaOH, and again just turned to the acid side, after which the reducing power was determined. Although it seems to be the general practice to clarify these solutions, it is not being done in

this laboratory for the one reason that analyses made on aliquots, some of which were clarified and others not clarified, gave no difference in reducing power. It may be necessary to clarify when the gravimetric method is employed, but not when the volumetric method is used.

c. *Hemi-celluloses.*—The residue obtained from the starch filtration was washed off the filter paper with 2.5 per cent. H_2SO_4, so that approximately 400 cc. of the acid were used. The mixture was then placed on a boiling water bath and allowed to remain there for two and a half hours, filtered, neutralized, turned acid, made to volume and analyzed. Here, again, the solution was not clarified.

It may be well here to make a few remarks regarding this almost entirely unknown group of carbohydrates, the hemi-celluloses. In a general way, it is known that pentosans (such as the xylans and arabans), hexosans (such as galactans, mannans, and glucosans), and such mixed polymers as the pectic bodies and mucilages, fall into this group. Beyond this practically nothing is known. In later papers, it will be shown rather definitely that this group is actively concerned with the normal nutrition of plants, and furthermore varies under some conditions of environment as much as starch. The water-holding capacity of all of the members of this group is of sufficient importance to warrant a detailed study of the group. The unfortunate thing about the reports regarding this group is that there are nearly as many methods of extraction as there are reporters. It is true that only relative results can be obtained, but until chemical methods are devised for the quantitative separation of this very important group of reserves, it will be best to adopt a uniform means of extraction, so that one worker's results may be compared with those of another.

C. Nitrogen.—a. *Nitrate nitrogen.*—Nitrate nitrogen determinations were made on aqueous extracts from three-gram samples of dried material. Devarda's method was employed.

b. *Total organic nitrogen.*—Total nitrogen contents were determined according to the Official Kjeldahl method, using three-gram samples.

D. Ash.—Ash determinations were made on two-gram samples. The dried material was placed in alundum crucibles, thoroughly mixed with 10 cc. of glycerol alcohol (1:2), and incinerated until subsequent weighings showed no further losses.

Conditions of the experiment

Three series of peas were run. Series 1 was started on the 29th of January, 1926, and discontinued March 20. Series 2 was used as a check on series 1, and was run from February 4, 1927, until March 24. Series 3 was begun immediately after the second series was completed. It ran from March 26, 1927, until May 24. Light from eleven 200-watt lamps was pro-

vided for series 1 and 2 from four o'clock in the afternoon until midnight of each day. Series 3 was grown under the normal conditions of light. In other words, series 1 and 2 were grown under long day conditions while series 3 was grown under a shorter light exposure; an exposure, however,

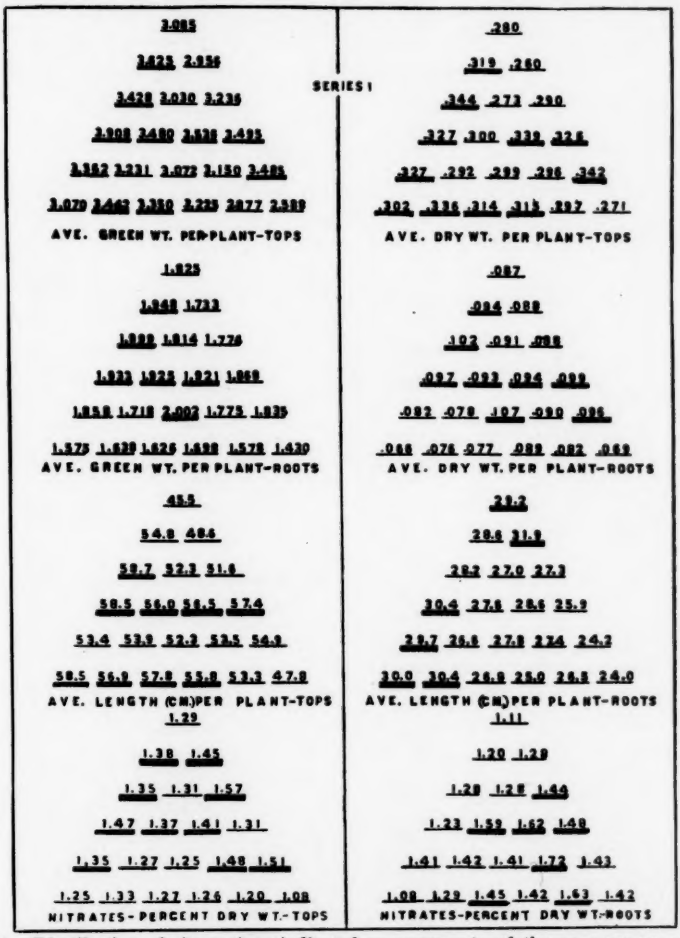

FIG. 1. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

that approached the normal day for the growth of peas. The temperature of the greenhouse was kept around 20° C. and the humidity remained relatively low.

As the reader looks at the triangles shown in figs. 1–6, the left hand corner represents the high $MgSO_4$ corner; the right hand corner the high

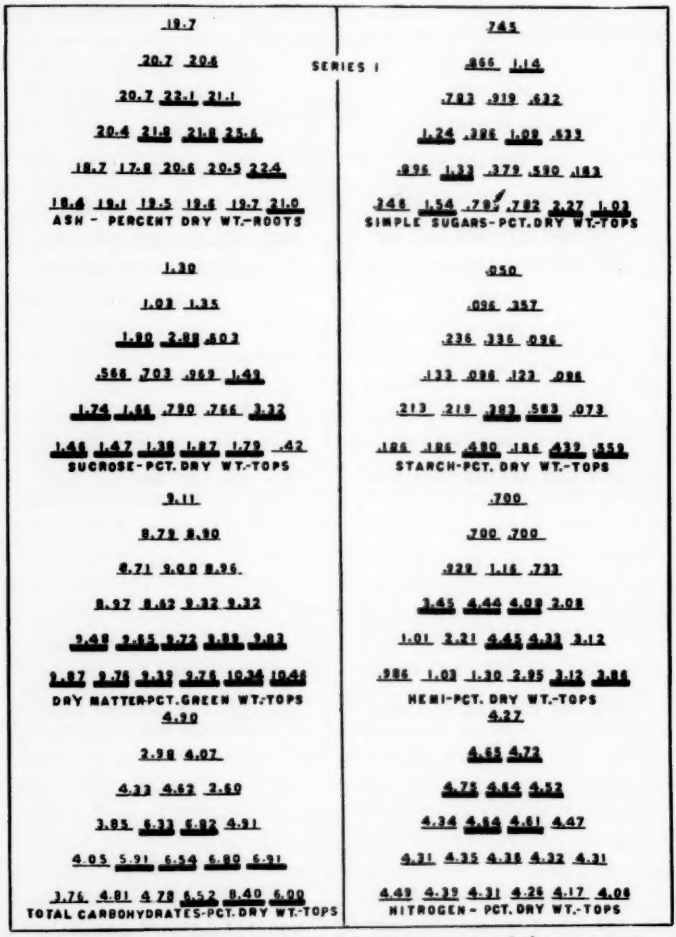

FIG. 2. Distribution of the various indicated measurements of the peas grown under a long light exposure from January 29, 1926, to March 20, 1926.

$Ca(NO_3)_2$ corner; and the top represents the high KH_2PO_4 corner. The heavily underscored figures indicate the cultures which produced plants highest in that particular measurement.

Results and discussion

It will be interesting to compare the results of series 1 and 2. These were really checks on one another and should compare favorably. When

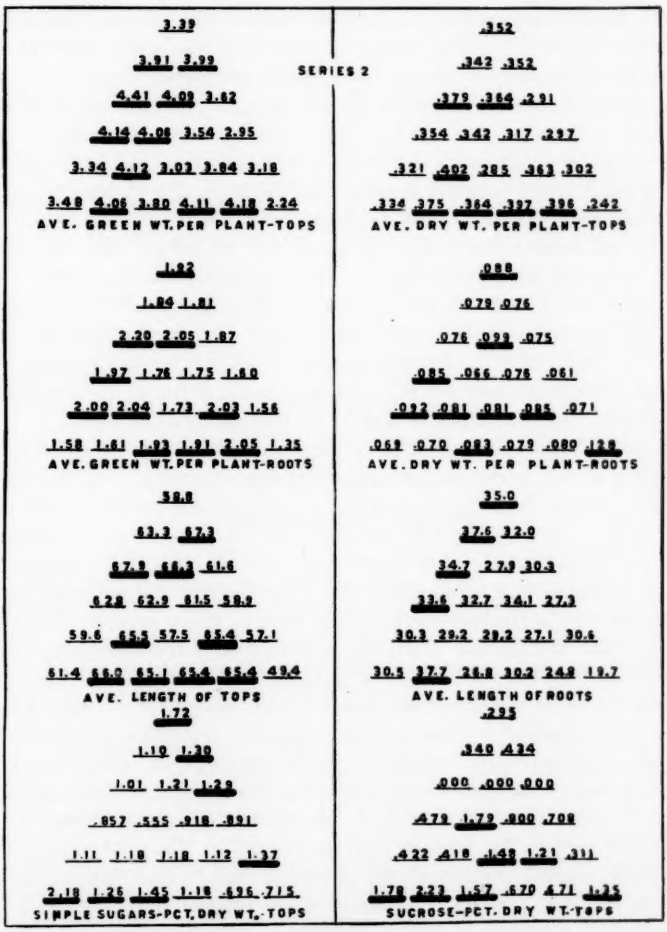

FIG. 3. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

one considers the length of the tops and roots of the two series (figs. 1 and 3), it is doubtful whether or not he would be favorably impressed

since there is a lack of similarity and yet there seems to be a tendency for the highest plants to fall in the same side of the triangle, that is, on the left hand side. When the green weights are examined (figs. 1 and 3), there is a much closer resemblance between the two series, but even they do not check in the way one would like to have them. Dry weights, likewise, tend to be the highest in certain regions and yet they are quite different. This difference may be due to the fact that there were more cloudy days in the period in which series 1 was growing than in that in which the second series was run. It thus becomes obvious that a different means of measuring the plants must be employed. Nitrate determinations (figs. 1 and 3) reveal little in the way of tangible results. In the tops, the highest nitrate contents were found more or less scattered over the triangle. This is to be expected since there are several things which would account for the variability, such as rate of absorption, rate of assimilation, etc. The nitrates of the roots seem to be most abundant in the high $Ca(NO_3)_2$ corner, and this might be expected, but surely this cannot be a measuring stick.

As the next attempt, the ash content of the roots was determined, and although the highest ash content centered on six cultures, it is still a question whether this would indicate good or inferior plants (fig. 2).

Sugar determinations seem to be more promising (figs. 2 and 3). Simple sugars do not yield any definite information except that little can be determined from them. This will be emphasized even more in a report to follow later. Sucrose likewise is erratic, although it shows a strong tendency to be highest in the lower part of the triangle. Starch (figs. 2 and 4) behaves in a similar manner in both series. Hemi-celluloses, too, seem to be most abundant in the lower part of the triangle. Total carbohydrates, that is, the sum of all the carbohydrates reported plus dextrins, are decidedly localized in the lower part of the triangle, and what is more, *they are the most abundant in plants grown in solutions which have the highest proportion of nitrates.* This, indeed, is a startling finding.

When total nitrogen determinations of the tops are examined (figs. 2 and 4), an extremely interesting thing is found. The highest amounts of organic nitrogen, on a per cent. basis, show an extremely well localized area, not where there is the highest nitrate content in the solution, but where there is the highest content of the potassium salt. This finding bears out the study of STOKLASA (**12**). A study of the relation of potassium to nitrogen assimilation under various exposures of light would, indeed, be fascinating. This is especially promising in view of the so-called radioactivity of potassium.

To show further that this question hinges around the nitrogen reserve, it will be well to call attention to another fact. With the gradual variation in the salt combinations used in any one row in the triangle, there must be

one culture which shows better salt absorption than any other culture in the same row. Thus, no matter how good or how poor a series of plants may be there must necessarily be one plant in this series which is either the best or the poorest. So in any row of the triangle one culture must either be the lowest in total nitrogen or the highest. With the gradual variation in the combination of nutrient salts there ought to be a gradual variation

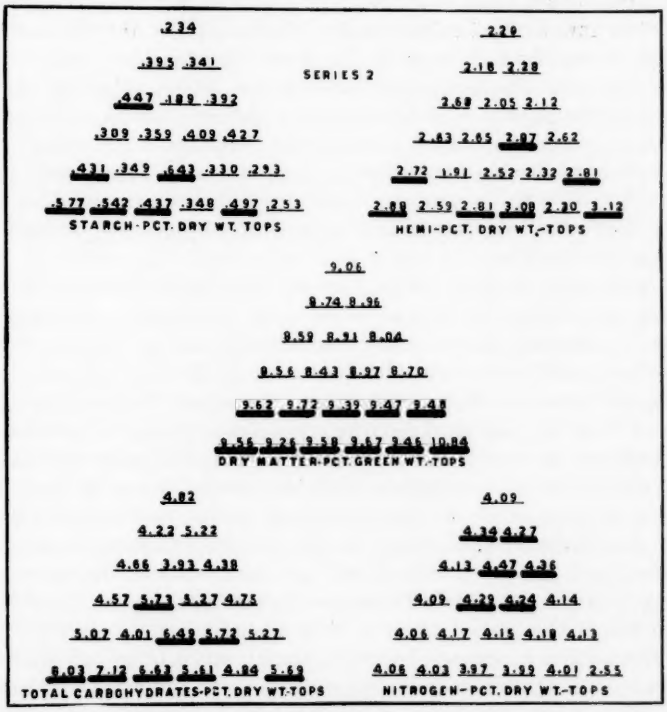

FIG. 4. Distribution of the various indicated measurements of peas grown under a long light exposure from February 4, 1927, to March 24, 1927.

in the total nitrogen contents in either one or two directions. This is especially outstanding in the total nitrogen results of all three series. Thus, the total nitrogen per cent. of series 1, the lowest line, reads 4.49, 4.39, 4.31, 4.26, 4.17, and 4.06. The row leading from the lower left hand corner to the upper corner reads 4.49, 4.31, 4.34, 4.75, 4.45, and 4.27. In the first row mentioned, the highest nitrogen assimilation occurred in the first culture and a gradual but definite decrease is found throughout the row until the last culture. In the second row given, the cultures with one exception in-

creased up to 4.75, and then decreased. It is extremely interesting to compare these trends in series 1 with those in series 2. Although all of the cultures ran lower in total nitrogen in series 2 than in series 1, these trends within the series show marked similarities. Thus in series 1, the high culture in the row on the left side of the triangle was the fourth culture up while in series 2 it was the fifth. In the row on the right, the fifth culture up was the highest while in series 2, the fourth culture was the highest. In the bottom row, the first culture on the left was highest, the one on the right was lowest, exactly as in series 2. It seems that these data show very definitely that the absorption and assimilation of nitrogen are markedly affected by the physiological balance of the culture solution. The trends in series 3 reveal the fact that a shorter light exposure very markedly affects the position of the highest culture in any one row. This seems to yield results favorable to the question asked by NIGHTINGALE and KRAUS (9): "Does light affect the fertilizer requirements of plants?" It seems very apparent that it does.

In a measure, now, we are able to generalize regarding the best culture solution, and regarding what we might term as the best plant or series of plants. KRAUS and KRAYBILL working with the tomato found a very definite relation existing between abundant organic nitrogen and succulence of plants, and between a high carbohydrate content and high dry matter. As either of these two groups of reserves became excessive, the plants no longer were regarded as normal because their reproductive process was hindered. When the plants were extremely high in nitrogen, many of their flowers failed to develop, while the general stature of the plant continued to enlarge. On the other hand, when the plants were extremely high in carbohydrates, some flowers developed and set fruit, but the vegetative growth of the plants was not sufficient to make efficient reproduction possible. Reproduction of the highest order was found only in those plants in which there was neither an excess of nitrogen nor an excess of carbohydrates. In other words, if these two reserves are moderately abundant in plants, the plant will express that internal condition by having vigorous vegetative development accompanied by fruitfulness. This, obviously, is what will be a normal plant. Although the tomato is a highly selected plant which must depend on artificial care for its proper development, it afforded a picture which has been verified time and again with other plants. The pomologist finds the same principles applicable to his fruit trees and regulates the reserve balance by proper pruning, fertilizing, etc. The florist practices it when he continually re-pots his plants until a short time before he wants them to flower. Then, he allows the plants to become "pot bound." This is nothing more than limiting root development and nitrate absorption and hence, correcting the highly vegetative state of his plants. The agronomist

realizes the application of the nitrogen-carbohydrate principle when he criticizes a field of grain as being too heavy in straw (too vegetative) or being too short (non-vegetative). The production of grain obviously is optimum when the straw is neither short nor long but intermediate. Thus, it seems only fair that this same criterion should be used in experimental plots and culture solutions.

In the triangles of series 1 and 2, the high nitrogen area is found in the top of the triangle, while the high carbohydrate area is found at the bottom. In view of the relation described above, it becomes obvious that the best plant of the series will not be found in the high nitrogen area, nor in the high carbohydrate area, but must be in a region which is intermediate with respect to these two groups of reserves. This region in the triangle, of course, will be between the high nitrogen and high carbohydrate areas. It was indicated above that plants high in nitrogen are high in moisture and those low in nitrogen and high in carbohydrates will be high in dry matter. A glance at the triangles representing dry matter in series 1 and 2 will not only verify this generalization but will show how closely the two series resemble one another.

The fact that the areas in which we find these reserves in greatest abundance are practically the same, for the first two series show rather decisively that here is a measuring stick which can be used to determine the region of the triangle in which best growing conditions prevail.

Thus, it is readily seen that this new criterion is far more satisfactory in determining the best cultures. Neither the total nitrogen nor the total carbohydrates can serve as indicators when taken alone, but when they are used in relation to one another, very definite information is obtained. Each maintains a definite area in series 1 and 2, and in view of the discussion here presented the best balanced plant cannot be found in either groups, but in the region between these areas. After this region is located, then such indices as length of the plants, their weights, etc., can select the best culture. Following is a diagram showing the numbering system:

21

19 20

16 17 18

12 13 14 15

7 8 9 10 11

1 2 3 4 5 6

In series 1, cultures no. 13, 14, 16, 17, 18, 19, 20 are eliminated from further consideration because of their high nitrogen content. Cultures no. 4, 5, 6, 8, 9, 10, 11, 13, 14 are eliminated because of their high carbohydrate content. Thus, cultures no. 1, 2, 3, 7, 12, 15, and 21 remain. Culture no. 21 is decidedly mediocre in its external appearance, being the shortest plant of the entire series. Of the remaining cultures no. 12 seems to be superior to the others in that it is high in green weight and dry weight of both tops and roots. Its tops are taller than those of any of the remaining cultures.

In series two, which should approximate series one since light conditions were practically the same, the carbohydrates are most abundant in the lower part of the triangle while the high nitrogen plants are found near the top. Thus, cultures no. (fig. 4) 13, 14, 16, 17, 18, 19 and 20 are eliminated because of their high nitrogen content. Cultures no. 1, 2, 3, 4, 6, 9, 10 and 13 are eliminated because of their high carbohydrate content. Cultures no. 5, 7, 8, 11, 12, 15, 21 remain for further consideration. It is to be noted that of these, 7, 12, 15 and 21 were also found in the remaining group in series 1 after the poorest cultures were removed. Thus, it is possible to select a culture which showed good growth in both series. That there is some difference in the growth of the plants can well be explained by temperature and humidity conditions. Culture no. 21 shows strong tendencies to favor high carbohydrate formation in both series. Culture no. 15 is decidedly inferior in its external features in series 2. Culture no. 12 is superior to no. 7, in green and dry weight of tops and in length of tops and roots, and since no. 12 showed a decided advantage over the other cultures in series 1, it seems only just to select it as the best culture in which to grow peas under a long day exposure. This balance is made up of three parts of KH_2PO_4, one part of $Ca(NO_3)_2$ and four parts of $MgSO_4$.

Now it will be of interest to see what effect a shorter day has on the relative positions taken by the highest carbohydrate and highest nitrogen cultures. Series 3 was run for that purpose. Beginning with the superficial measurements such as length of tops, roots, their green and dry weights (fig. 5), it is seen at once that our biggest plants are in an entirely different part of the triangle than they were in the previous series. Furthermore, when the region in which nitrogen is the highest is examined (fig. 6), it is seen at once that it has in a sense migrated to a lower position on the triangle nearer to the high nitrate corner. How has the high carbohydrate area responded? That has seemingly been switched around so that now instead of being in the lower right hand corner as in previous series, it is found in the left hand side of the triangle. Under these conditions, we must look for the best plant in a place different from that of the other series.

In applying the elimination process to this series (fig. 6), cultures no. 4, 9, 10, 13, 14 and 16 represent high nitrogen cultures. Cultures no. 1, 2, 3, 7, 12, 16, 17, 18, 19, 20 and 21 represent high carbohydrate cultures. Thus, the best plants must be found among cultures no. 5, 6, 8, 11 or 15. Culture no. 6 does not compare favorably with the other cultures so far as the external measurements are concerned (fig. 5). Culture no. 8 is rather

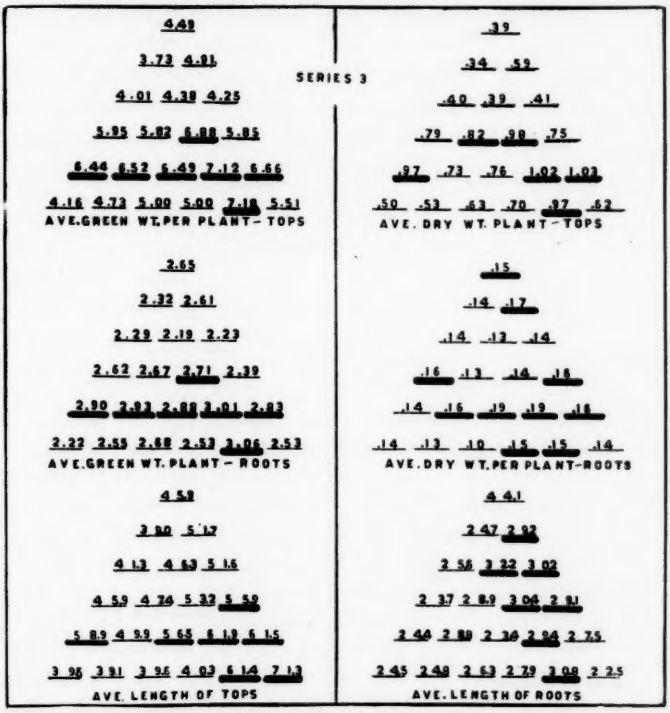
SERIES 3
AVE. GREEN WT. PER PLANT – TOPS
AVE. DRY WT. PLANT – TOPS
AVE. GREEN WT. PLANT – ROOTS
AVE. DRY WT. PER PLANT – ROOTS
AVE. LENGTH OF TOPS
AVE. LENGTH OF ROOTS

FIG. 5. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

high in moisture while culture no. 11 is higher in dry matter than any other culture. Culture no. 15 shows a tendency for greater root development in proportion to tops and thus culture no. 5 is left. It is moderately high in dry matter and nitrogen. Its low content of carbohydrates is well explained by the fact that its top and root development is excellent. Thus, culture no. 5 apparently contains the optimum balance of salts in which to grow peas under an intermediate light condition. This balance is represented by one part of KH_2PO_4, five parts of $Ca(NO_3)_2$, and two parts of $MgSO_4$.

This, perhaps, helps in a measure to explain some of the inconsistencies which are encountered in the literature regarding the plants grown in the triangular system. We are contending not only with a variety of salt combinations but also with the way plants respond to these combinations in building their reserves. These reserves then seemingly dictate the external expression of the plant. Further than that, we are contending with the

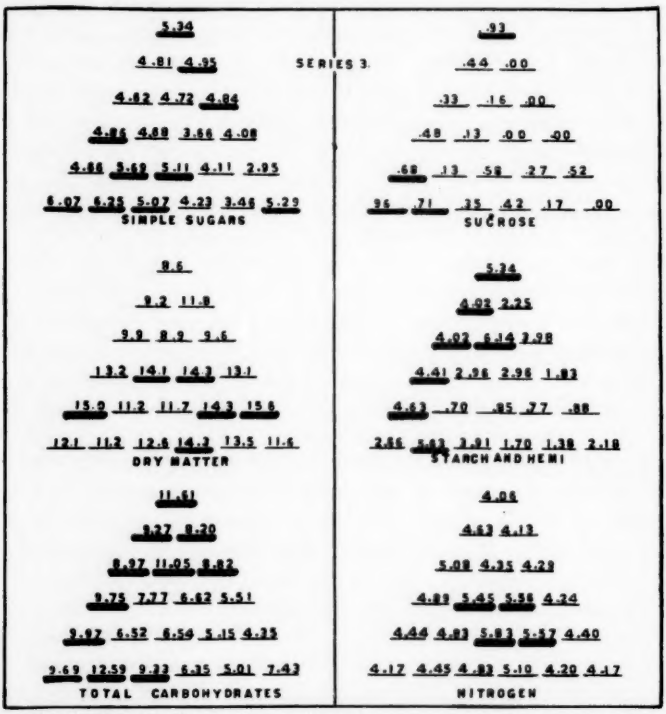

FIG. 6. Distribution of the various indicated measurements of peas grown under a shorter light exposure from March 26, 1927, to May 24, 1927.

effect that light has on modifying the response of plants to the combination of salts.

Following such studies it becomes apparent that the triangular system has the extremely important rôle in plant physiology of showing the effect that light has on modifying the fertility requirements of plants in the various sections of the country and at various seasons of the year. Furthermore, the aid of the triangle will be of inestimable value in studying the effect of the various elements on such functions as nitrogen assimilation and carbohydrate accumulation and utilization.

The writer wishes to express his gratitude to Dr. E. A. Bessey for his criticisms of the manuscript, and to Dr. R. P. Hibbard for the use of his laboratory and equipment.

Summary

1. Three series of peas were grown in the triangular system of water cultures. Series 1 and 2 were grown at the same time of the year, the former in 1926, the latter in 1927. Series 3 was run in 1927 but at a date later than series 2.
2. From data presented, it is obvious that such criteria as length of plants, green and dry weights, etc., cannot give a correct idea of the best balance of salts for the growth of plants.
3. Indices such as highest ash content and highest nitrate content are presented, but are shown to be erratic in their positions and hence unreliable.
4. This index unreliability is likewise true of the soluble forms of carbohydrates.
5. Data are presented which show that KH_2PO_4 has a very marked influence on nitrogen assimilation.
6. The areas in which nitrogen and carbohydrates are highest, respectively, assume very definite positions in the triangle.
7. The positions of high nitrogen and high carbohydrate areas are taken at opposite sides of the triangle and leave an area in which the best plants of the triangle may be expected to be found.
8. It is shown that a shorter exposure to light results in different positions being occupied by the best cultures. This must be expected in view of the behavior of the two large groups of reserves found in the plants which were grown under shorter light exposures.
9. A possible rôle of the triangular system of water cultures in future research regarding fertilizer requirements is mentioned.

Michigan State College,
East Lansing, Mich.

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ANATOMICAL MATERIAL FOR THE STUDY OF GROWTH DIFFERENTIATION IN HIGHER PLANTS*

RAY BOUILLENNE

(WITH THIRTEEN FIGURES)

As Fauré-Fremiet[1] says very explicitly in his introduction to "The kinetics of development," the morphological study of ontogenesis of the organs and the order of their developmental succession in the individual during the formative period represents a merely external point of view; it offers merely a point of contact. The author does add, however, that morphological indications are as yet of too great importance to be completely neglected.

Our physiological studies on one hand, and certain anatomical investigations on the other, made during a botanical expedition[2] in the Brazilian Amazon, have by their coincidence made it possible for us to furnish anatomical material for a study of growth differentiation.

We wish to draw attention to a singularly interesting case which illustrates that morphological considerations are able to bring us to the same point as purely physiological results are doing.

Physiological studies of growth phenomena have permitted us to perceive the relations between growth and age, between growth and the chemical composition of the plant organs and the correlations existing in the development of the different organs. It has proved possible to trace curves expressing mathematical laws. These have the same general trend in plants as in animals. The majority of authors have recorded their observations in successive measurements of weight, of volume and of length during the course of development; for example, the works of BRODY[3] on the growth of the domestic fowl, BRODY and RAGSDALE[4] on the cow, and those of PRIESTLEY, PEARSALL and EVERSHED[5,6] on *Tradescantia* and other plant roots.

* Translated from the original French manuscript by Dr. MARY JUHN, research assistant in zoology, the University of Chicago.

[1] FAURÉ-FREMIET, E. La cinétique du développement. Les Presses Universitaires de France. Paris. 1925.

[2] Belgian botanical expedition in Brazil, 1922–1923, organized by the Belgian Government and the "Fondation Universitaire."

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[4] ———, and RAGSDALE, A. C. The rate of growth of the dairy cow. Jour. Gen. Physiol. 3: 623–633. 1921.

The observations at our disposal on the morphological variations correlated to these modifications of weight, of length and of volume, though of the greatest importance, are very few in number. In zoology, for example, descriptions of embryonic developmental phases and of metamorphoses have been furnished since a long time. To-day the attempt is being made to find the physico-chemical reasons for the processes of differentiation and a distinction is made between the part due to heredity and the part due to actual causes (causative embryology). Valuable arguments in histology and in cytology have been furnished by CONKLIN'S paper on the nucleo-cytoplasmic relation during the course of development. These findings have been amplified by ROBERTSON in his book, "Chemical basis of growth and senescence."

Botanical literature, however, appears to contain little information concerning the different morphological stages through which a plant passes during its developmental period. I do not at all wish to discuss the adaptations or accommodations to actual conditions such as have, for example, been described by MASSART for *Polygonum amphibium,* but rather to consider the variations in structure during the normal developmental period. The reason for this is that plant embryology can only be considered from a very special point of view, and this point of view differs considerably from the one used in animal embryology.

Thus metazoan embryology ends with the recognition of a young individual whose general organization is, so to speak, established and which will subsequently undergo only modifications of size, of maturation, and senescence. In the plant on the contrary, the stage of embryonic differentiation does not terminate with the formation of the embryo and the ripening of the seed. The meristematic tissues of the stems and the roots of plants continue to function as generative tissues during the entire life of the plant; there is a kind of prolonged embryonic condition which is confined to certain regions, at the extremities of stems and roots, and to the cambium. These meristems appear to be exposed at every moment of the plant's life to the internal conditions of heredity, ontogenetic evolution and to the external conditions of the environment.

These generative tissues, but undifferentiated ones, give rise to primary tissues within which structures become established; and so appear new regions of stems and roots.

We know that every part of a stem, for instance, has its own structure, varying within the limits of the species. Are we able to conclude from these variations that meristem has different potential qualities?

[5] PRIESTLEY, J. H., and PEARSALL, W. H. An interpretation of some growth curves. Ann. Bot. **36**: 239–249. 1922. (See also Ann. Bot. **36**: 485–488. 1922, and **37**: 261–275. 1923.)

[6] ————, and EVERSHED, A. F. C. H. A quantitative study of the growth of roots. Ann. Bot. **36**: 225–237. 1922.

The question proposed in this paper is as follows: *Are there any morphological proofs for different potentialities of the meristem during the normal growth phenomena? Is it possible to find structures whose succession, along a stem or a root, is parallel to the physiological variations of growth?*

Our intention is not to make a study of the relations between the physicochemical phenomena of growth in plants and their forms and structures but rather to add new material to the knowledge of these correlations; in other words to give these latter an interesting morphological illustration.

Description of material

Up to the present time, the leaves, stems, branches and inflorescenses have been the only parts of higher plants to furnish information on the subject of correlations between growth and structure. As to roots, they generally show no important variations; they have a very uniform anatomical structure in all vascular plants and this structure is identical or constant along the long axis. In the Monocotyledons as well as in the Dicotyledons the root consists of a central cylinder, forming in cross-section a perfect circle, limited at the external periphery of the central cylinder by an endodermis and a pericycle. On this curve is oriented a large number of vascular bundles, and the whole is surrounded by the cortical parenchyma. In the young stages one can scarcely distinguish the root of Monocotyledons from those of Dicotyledons except perhaps by the greater number of vascular bundles in the former. In the adult Dicotyledon a cambium tissue modifies the structure. The Monocotyledons which have no cambium retain their diameter and primitive organization.

We have had occasion to study from a purely anatomical viewpoint a very interesting case of variation, the "stilt" roots of the Amazon Palm, *Iriartea exorrhiza* Mart. We believe it will be interesting to present the facts, freed from the purely anatomical considerations.[7]

Iriartea exorrhiza Mart., a ceroxylian palm, lives in the forests of Varzea[8] along the banks of the Amazon in very muddy soil which is inundated during the greater part of the year. The palm, fig. 1, has large, stilt-like aërial prop roots which take their origin from the stem at different nodes and which enter the soil at an oblique angle. This arrangement constitutes an unique case among the palms and is very rare among the Monocotyledons.

We will describe the structure of these roots in young palms of different ages and in the adult; this will show the different peculiarities of root structures from germination.

We find that the roots are inserted higher and higher up on the lower region of the stem, and that the most recently formed aërial roots in the

[7] BOUILLENNE, RAY. Les racines-échasses de *Iriartea exorrhiza*. Mem. Acad. Roy. Sci. Belgique. 1925.

[8] Varzea = alluvial prairies or forests.

adult palm are given off at more than two meters from the soil. The roots are not all of the same diameter. Those inserted nearer the soil have a smaller diameter than do the upper roots.

FIG. 1. *Iriartea exorrhiza* Mart. (Palm), from the Botanical Garden, Rio de Janeiro, showing the prop or stilt roots which arise at different levels from the base of the stem. The inflorescence is visible beneath the leaf sheaths at the apex of the stem.

I. Young plants

The entire series of observations made can be demonstrated from two of the numerous young plants that we collected in the Varzea forests. We

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end.
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1m

2.

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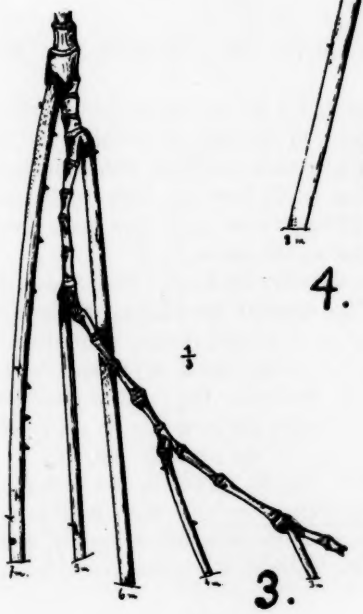
7m

4.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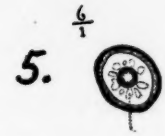
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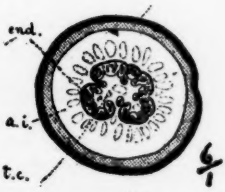
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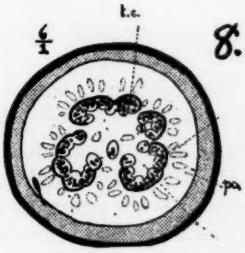
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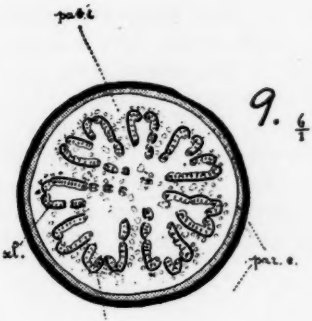
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9.

FIG. 2. Base of a young plant, 40 cm. tall, showing five adventitious roots, inserted at different levels on the stem, and having respectively from lowest to highest 2, 3, 3, 4, and 5 mm. diameter. Magnification, ⅓.

FIG. 3. Base of a young plant of *Iriartea* showing the increasing diameter of the "stilt" roots with higher insertion on the stems.

FIG. 4. Portion of a young plant, one meter tall, showing five of its adventitious roots with 6, 7, 8, 9, and 10 mm. diameter respectively. Drawn ⅛ natural size.

FIG. 5. Cross-section of a primary root of *Iriartea*, 2 mm. in diameter. See description in text.

FIG. 6. Cross-section of a root 4 mm. in diameter. Thirty-one xylem bundles. See text. l = aerial lacunae.

FIG. 7. Cross-section of a 6 mm. root, showing 62 xylem bundles. See text. end = endodermis.

FIG. 8. Cross-section of a slightly larger root, showing the same features as fig. 7, but intercellular spaces more pronounced. t.c. = conjunctive tissue.

FIG. 9. Cross-section of a 10 mm. root, having 229 xylem bundles. See text for description.
par. e. = external cortical parenchyma.
par. i. = internal cortical parenchyma.
scl. = sclerenchyma.

will therefore limit our description to these two, the youngest and the oldest, of the entire lot.

The youngest plantlet (fig. 2) measures 0.4 m. in length and has three leaves, the narrow stem having a diameter of 2.5 mm. at the base and 10 mm. at the apical end. The diameter increases regularly from the base to the tip. The basal part of the stem itself has two little roots, one of which is the principal root. Adventitious, aërial roots have been given off singly at some of the nodes, quite close to the leaves.

These aërial roots appear therefore at different levels; they cause an extensive rupture of the epidermis a little beneath the nodes. These five aërial roots have diameters measuring 2, 3, 3, 4, and 5 mm., respectively. These measurements have a direct relation to the diameter of the stem at the point of their insertion, in other words, the nearer the point of insertion of the aërial root to the apical end of the palm, the larger is its diameter. See table I. This observation is equally true of the plantlet (fig. 3).

The oldest of the lot of young plants (fig. 4) measures one meter in length. The diameter of the stem in the region near the leaves is 20 mm.; the five aërial roots formed in this region measure from below up 6, 7, 8, 9, and 10 mm. in diameter. As one can see, there is a regular gradation in

the thickness of these roots. This observation is confirmed by data from other plants, which will not be presented here, but which are incorporated in the graphic data of fig. 10 and table I.

TABLE I

DIAMETER RELATIONS OF ROOTS AND STEMS

DIAMETER OE ROOTS	DIAMETER OF STEMS	NODES COUNTED FROM PRINCIPAL ROOT UP
mm.	mm.	
2.0	2.5	1st
3.0	3.0	2–3rd
4.0	4.0	7th
4.5	.....	.....
5.0	5.0	9th
6.0	6.0	16th
7.0	7.0	.....
8.0	9.0	.....
9.0	9.0	.....
10.0	10.0	.....

It is by no means possible to demonstrate sharply this correlation of stem and root diameters on larger parts of stems and on adult plants. On the one hand, there is a greater number of roots appearing at nearly the same point and modifying the true diameter of the stem; on the other hand, roots are lacking on the upper parts of stems.

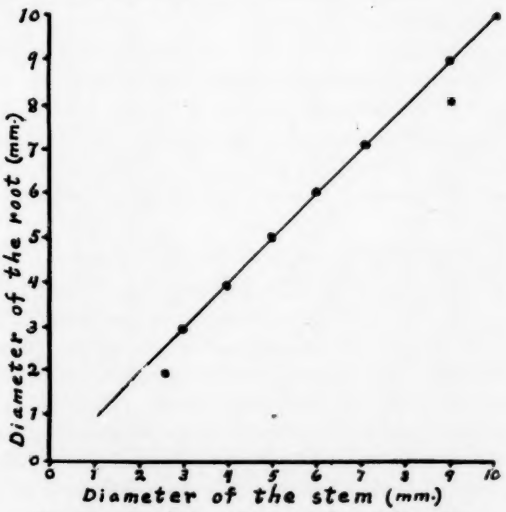


FIG. 10. Correlation of stem and root diameters.

Anatomy of the different roots of the young plants

Cross-sections were made through the roots of these two plants at a distance of 5 cm. from the stem. This level corresponds to the aërial parts. The narrowest root, the primary root, is 2 mm. in diameter and has a true central cylinder, forming a *perfect circle and having 12 xylem bundles alternating with 12 phloem bundles.* The structure of this root can in no wise be distinguished from that of a normal Monocotyledon (fig. 5).

The root measuring 4 mm. in diameter and inserted at the seventh node, approximately at a distance of 10 cm. from the soil, reveals an extremely interesting condition of the central cylinder. 1. The central cylinder is larger than that of the root just described; it has 31 xylem bundles and 33 phloem bundles. 2. The peripheral outline of the central cylinder is wavy. 3. The central cylinder is ruptured (fig. 6).

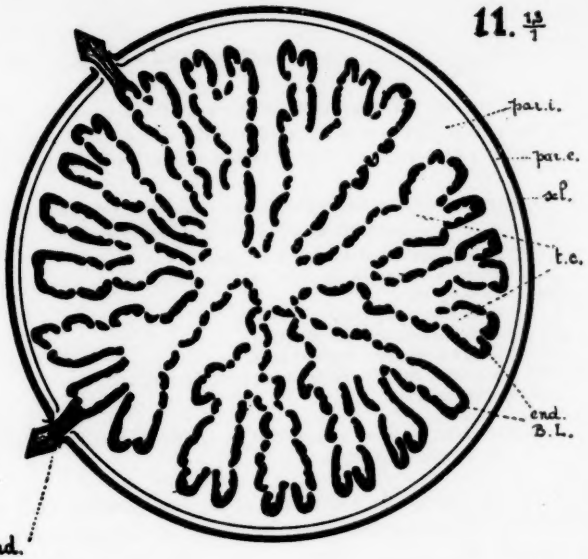
The root measuring 6 mm. in diameter (fig. 7) and inserted in the plantlet at the sixteenth node, at a distance of about 20 cm. from the soil is characterized by the same three differences described for the 4 mm. root, but in a more marked degree. 1. There are 62 xylem bundles forming a yet larger central cylinder and 63 phloem bundles. 2. The wavy outline is even more distinct, and the undulations are of different sizes. The most important ones, five in number, take the form of lobes, within whose curves the xylem bundles are oriented. These five lobes are wavy in outline as well. 3. The central cylinder is ruptured in several places so that it appears as if divided into segments. There are five segments corresponding to the five principal lobes, the edges of these segments are more or less separated from each other by parenchyma (fig. 8).

The root measuring 10 mm. in diameter and inserted in a thick node at 50 cm. from the soil shows the following arrangement: 1. There are 229 xylem bundles. 2. The central cylinder is deeply lobed. Each of these lobes is itself bi- or trifurcate, and these secondary lobes are also separated from each other by strands of parenchyma. The lobes themselves are ruptured in several places, giving the appearance of segments of different sizes.

The large roots of the adult plant

The large aërial roots of the adult *Iriartea* (figs. 11 and 12) are inserted very high up on the stem and their diameter varies from 50–60 mm. This diameter remains approximately constant in the entire aërial region. A cross-section through such a root shows a peculiar design, occupying the entire section, a kind of star with blunt rays. These lobes, of different sizes, are separated from each other by deep sinuses, and are themselves formed by smaller secondary and tertiary lobes. The contour of the lobes is not continuous but broken up into numerous segments of various sizes.

11. $\frac{13}{1}$



12. $\frac{3}{1}$

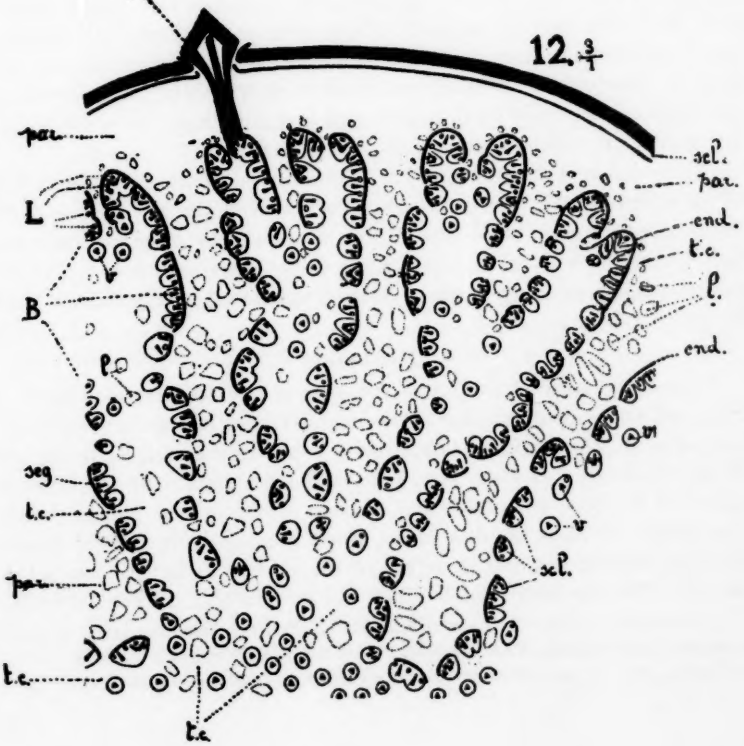


FIG. 11. Cross-section, ×1.3, of root of adult *Iriartea exorrhiza*. Diameter, 5 cm. The central cylinder is visible, and shows the star-shaped outline formed by its lobes and segments. Two thorns, sclerified radicles, may be seen.

FIG. 12. Single lobe of the central cylinder, ×3. One can distinguish the elements constituting the lobes and segments.

B = xylem, indicated by black arrows.
L = phloem, indicated by small white circles.
end. = endodermis.
l. = intercellular spaces.
par-e. = external cortical parenchyma.
par-i. = internal cortical parenchyma.
rad. = sclerified radicle, forming a spiny projection.
scl. = sclerenchyma.
seg. = a segment.
v. = an isolated vessel surrounded by sclerenchyma; the vessel is indicated by a black triangle.

The star-shaped design is composed of xylem bundles that are arranged perpendicularly to its outline, each composed of 3–5 tracheae and one or two large vessels. The number of these xylem bundles is quite exceptional, being 1300–1500. The phloem bundles usually, though not always, alternate with the xylem and are present in even greater numbers, 1600–1800. To our knowledge such a complicated structure has been described only in the roots of *Iriartea*.[9] It is easily explained from transition stages revealed by the study of the young rootlets, although first studies on the adult plant did not lead to an understanding of its root nature.[10]

These different stages are not to be considered as stages in the evolution of the structure of a root in the course of its development. In the roots of the Monocotyledons, the diameter once attained, the structure once established, remains without change. Each of the roots as it is given off from the stem has a structure such as we have described, and retains it during its entire existence.

Discussion

I. In studying the structure of the roots of *Iriartea exorrhiza* Mart., one finds that the central cylinder shows modifications, lobing and breaking up into segments, the degree of complication being directly related to the width of the diameter. Another very remarkable peculiarity of the structural aspect of the root sections is the number of xylem and phloem bundles. The number of these bundles varies, being greater the larger the root and the more complicated the structure of the central cylinder. See tables I and II. The number of the bundles, the structure of the central cylinder

[9] BOUILLENNE, RAY. Les racines-échasses de *Iriartea exorrhiza* Mart. (Palmiers) et de *Pandanus* div. sp. (Pandanacées). Mém. Acad. Roy. Sciences Beligique. **8**: 1925.

[10] MOHL, ————. Historia naturalis Palmarum. Martius, vol. 1. 1823.

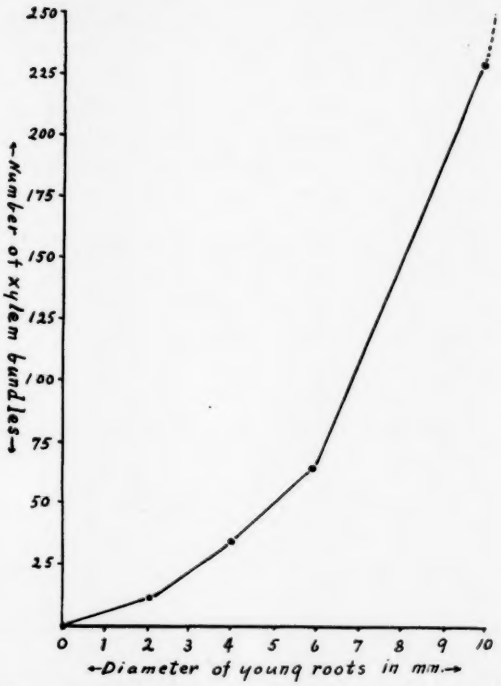


FIG. 13. Graphical representation of the relation of root diameter to the number of xylem bundles.

TABLE II

RELATION OF NUMBER OF VASCULAR BUNDLES TO ROOT DIAMETERS

NUMBER OF VASCULAR BUNDLES	DIAMETER OF THE ROOTS
	mm.
12	2.0
31	4.0
42	4.5
63	6.0
229	10.0
1300	50.0

and the increase in width of the diameter of the root are parallel to each other in their changes from one root to another.

II. When one follows the development of the plant from the beginning of germination, one observes that the first roots formed on the plant are

narrow and simple in structure, and that the roots which appear later during the growth period of the stem are more and more complicated; finally, that the most recently formed roots, which appear on the adult palm, are the largest and have the most highly differentiated structure. We find therefore, from node to node, from the basal to the apical end, all the transition stages between the typical Monocotyledon root to the aberrant root of the adult palm. The succession of these structures depends on growth phenomena.

III. These structures result from the functional activity of the meristematic tissue of the root, passing through a primary stage of tissue development. They appear at the outset with their peculiar structural features at the moment of the formation of the root. They are closely related to the height of insertion in the stem. In proportion to the growth and development of the stem, the meristematic tissues of the roots, which appear at different levels, participate in the stem ontogenetic development.

IV. We know already that a close relation exists between the structures of branches, leaves, and inflorescences, and the stem. A. The leaves arranged along the length of the stem of any annual plant from the base to the tip show the following arrangement: a. Cotyledonary leaves; b. primordial leaves, relatively simple; c. leaves that from node to node show progressive stages of complication until they achieve the typical form of the species considered; d. leaves situated beneath the inflorescences, which are reduced in size, and take the form of bracts.

B. The branches, arising from the development of the axillary buds, have a structure the pattern of which is influenced by that of the stem at the point of their insertion. One knows that the same meristem has formed stems and buds at a given moment; the stem continues to grow while the buds often remain in the resting stage until favorable circumstances arise to release their activity, in the formation of lateral branches. These lateral branches are at the beginning, larger and more complicated in structure when they are inserted on a part of the stem having a higher degree of differentiation and a wider diameter.

C. The inflorescences may be considered from the same point of view. The observations of GRAVIS[11] showed already a correlation between the region of the stem at which the inflorescences appear and the type of structure evolved at this level.

D. The stems themselves show modifications of their anatomy. According to the level, the number of fibro-vascular bundles varies, and so does their arrangement and their pathway through the node as well as the internode. The author previously quoted, studied the different types of

[11] GRAVIS, A. *Urtica dioica* L. Mémoires Acad. Roy. Sci. Belgique. 1896.

structure in the internodes of *Urtica dioica* and *Tradescantia virginica.* At the beginning of the growing period of a stem, the structure becomes successively more complicated, next it becomes uniform and finally undergoes a period of degradation or retrogression.

The meristem at the different nodes of a stem therefore did not function in a uniform manner. It has led to the formation of structures, varying around the specific type, according to the growth periods. These different modifications have for a long time attracted the attention of different workers; they have been attributed to factors of nutrition, to the influence of the surrounding medium. These factors certainly play a part, nevertheless it appears to us that it would be a mistake not to consider the evolution of the growth itself. It is generally said, that the meristem, for example that of a stem, is the youngest and the most active tissue of this stem. Since it is the generative tissue, it is as a matter of course the most active. But, borne at the tip of the stem, during growth, it functions since germination, and in the adult plant, at the extremity of the stem which it forms, it is the oldest tissue present. And this tissue will become still older, the more the stem will elongate over a long period. Therefore, even though it is a generative tissue, the meristem ages. A part of the organographic and anatomical modifications of plants appear to be attributable to this evolution of growth. The vigor of the meristem and the complication of the structures formed are on a par; they augment in proportion as the plant grows up to a maximum, after which there is a slowing down to winter rest or death. This illustrates the bell-shaped curve of the majority of the phenomena of development.

Conclusion

Our observations on the "stilt" roots of the palm, *Iriartea exorrhiza* Mart. make it possible to add a new element to these connections between the morphology and the physiology of development. They establish between *two vegetative organs, roots* and *stems,* anatomical correlations which appear to be due in part to the ontogenetic evolution of the plant. Under the conditions stated for *Iriartea,* where both these organs are formed at the same time from connected meristems, we offer the suggestion, that the parallel variations of structure may be explained by the modifications of the physiological conditions which surround the meristem in the course of development.

UNIVERSITY OF LIÉGE,
BELGIUM.

THE INFLUENCE OF ONE ION ON THE ACCUMULATION OF ANOTHER BY PLANT CELLS WITH SPECIAL REFERENCE TO EXPERIMENTS WITH *NITELLA**

D. R. Hoagland, A. R. Davis, and P. L. Hibbard

(WITH FOUR FIGURES)

We have previously published the results of experiments on *Nitella* cells, which emphasized the primary importance of illumination and of temperature in determining the accumulation[1] of halogens in the cell sap and at the same time a number of preliminary observations were reported with regard to certain inter-ionic effects in relation to the process of accumulation (**5**). This latter question had also been studied earlier in connection with experiments on the absorption of ions by barley plants (**4**). While it appears that the accumulation of solutes by a plant cell is dependent on the growth or metabolic activities of the cell, it is also clear that the magnitude of the actual accumulation under any given conditions of light and temperature is influenced by the concentration and composition of the culture solution. It is this latter aspect of the question that we now wish to discuss.

The general methods of experimentation heretofore described were used in the present work. *Nitella* cells were immersed in the solutions under investigation and at the end of the experiments the cells were thoroughly rinsed with distilled water and the larger cells (from 1–3 inches in length) were broken individually, the sap being collected in amounts varying between 1 and 25 cc. Since a single cell yielded only one or a few hundredths of a cubic centimeter of sap, each composite sample of sap collected represented a very large number of cells.[2] The method of Hibbard (**3**), specially developed for this purpose, was employed in analyzing the sap for its halogen content, which procedure made it possible to obtain results of sufficient accuracy even when dealing with very small volumes of sap. (Determinations were made in duplicate or triplicate.)

* Presented before the American Society of Plant Physiologists, Nashville, December, 1927.

[1] This term is used to indicate the process of concentrating solutes in the cell sap, with the attainment of a higher concentration inside than outside, as in the sense of Osterhout.

[2] It is to be noted that different lots of cells may differ in their physiological state, so that quantitative comparisons must usually be made within each experiment.

Since the earlier experiments had shown that the accumulation of halogens in the cell sap is dependent upon adequate illumination and that the process goes on relatively slowly in any case, we have conducted our preesnt experiments with reference to these conditions. In certain instances artificial light of considerable intensity was employed; sometimes the illumination was continuous. The exposure times extended in some instances to two weeks or more, a point especially to be noted, since it indicates that we were dealing with physiological processes involved in mineral nutrition, and not simply with degrees of permeability. When sufficient time is permitted to elapse a perfectly definite accumulation in the cell sap of certain mineral elements, sometimes of relatively large magnitude, can be demonstrated. If a very limited period of time were employed with similar solutions, it might appear that the cells were almost impermeable to electrolytes and thus the processes most vital to nutrition might escape detection.

The relation of concentration to accumulation

The first question to be considered is concerned with the relation between ionic concentrations in the culture medium and in the cell sap. This question was studied by determining how variations in the concentrations of Br ions in the culture medium influenced the accumulation of these ions in the cell sap. Several experiments were performed. In one experiment the accumulation was permitted to continue for one day only, while in another experiment the cells were kept in contact with the bromide containing solutions for over a month. The results of three different experiments are plotted in fig. 1, in which the curves are of a logarithmic type. Thus the factor obtained by dividing internal by external concentration has a much higher value for external solutions of low concentration than for those of high concentration. Special significance is attached to the present data, inasmuch as they reflect directly the conditions existing in the cell sap, but it is interesting and important to recognize that these experiments on *Nitella* cells are entirely consistent with those made on other plants by necessarily less satisfactory methods. Our own results using agricultural plants give evidence of the existence of similar relations between internal and external concentrations of electrolytes; and likewise consistent are the results on storage tissues reported by Stiles and Kidd (9). All of the investigations emphasize a principle which is highly important to the student of soils and plants, for the reason that in general the concentrations of essential elements in soil solutions are low, sometimes extremely low, and it is essential that land plants should possess a means of concentrating inorganic elements at a relatively high rate from solutions of low concentration.

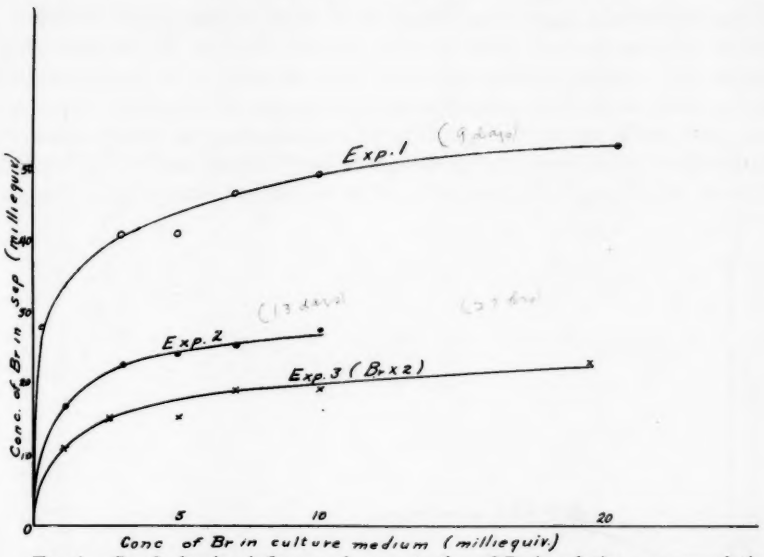


FIG. 1. Graph showing influence of concentration of Br in solution on accumulation of sap. Exp. 1, 9 days' duration. Continuous artificial illumination from two 300-watt lamps; temperature 23°–25° C. Exp. 2, 13 days' duration; diffuse daylight; room temperature. Exp. 3, 27 hours' duration; diffuse daylight plus continuous artificial illumination, two 300-watt lamps. Experiments 1 and 3, without buffer. Exp. 2 with phosphate buffer. Initial pH of all solutions, 5.0–5.4.

If so desired, the data under discussion could be fitted into one of the well known adsorption formulae, but it has not appealed to us that this method of interpretation assists very materially in explaining the mechanism involved, especially when we bear in mind the importance of the metabolic activities of the plant in connection with the ability to concentrate solutes in the interior of cells, as well as the evidence in support of the idea that the chemical elements in question exist in the cell sap primarily in dissociated form, and not for the most part adsorbed on organic compounds. Furthermore, the adsorption formula is of too general a character to serve as a guide to the understanding of such a highly complex series of physiological processes.

Effect of cations and anions on accumulation of Br ions

As another phase of the research we planned to determine to what extent the accumulation of Br ions could be modified by other ions of the same or opposite charge present in the culture solution. First referring to the influence of other anions, experiments were carried out with solutions

containing KBr to which were added KNO_3, KCl, K_2SO_4, or KI in various concentrations (figs. 2–3, tables I–II). The NO_3 and SO_4 ions had no effect whatever in retarding the accumulation of Br ions in the cell sap.[3] In fact the tendency was in the opposite direction, which we attribute to the accelerating influence of the increased K concentration, an effect definitely suggested by experiments to be discussed later in this paper. In marked contrast to NO_3 and SO_4 ions, Cl ions in equivalent concentration signifi-

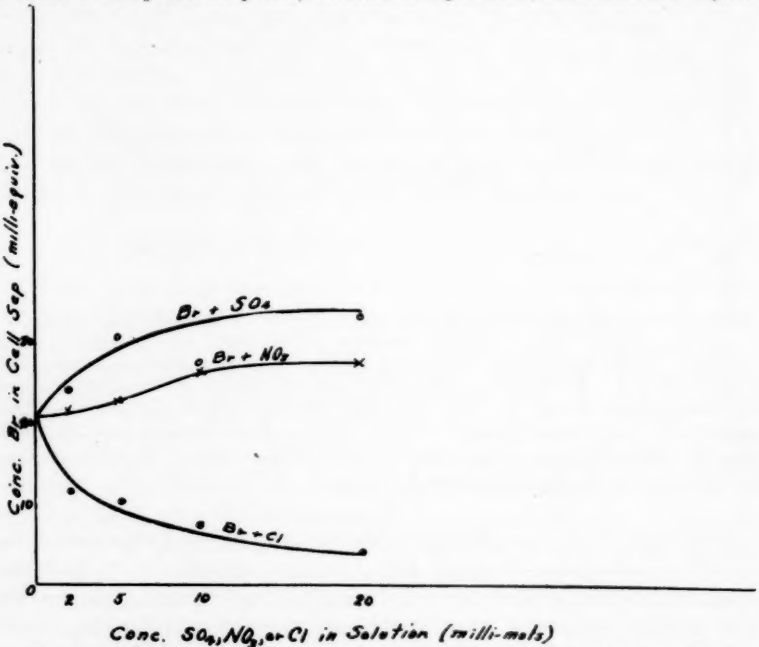


FIG. 2. Graph showing influence of K_2SO_4, KNO_3 and KCl in solution, on accumulation of Br in cell sap; 4 days' duration; continuous artificial illumination with two 300-watt lamps; temperature 23°–24° C. Initial pH of all solutions, approximately 5.0. Phosphate buffer solutions used, containing 0.005 M. KBr to which were added K_2SO_4, or KCl as indicated.

cantly retarded the accumulation of Br in the cell sap and I ions were also definitely effective in the same way, although not to the same degree as Cl ions. A pronounced retardation in the accumulation of Br ions occurred as a result of the presence in the culture solution of Cl ions in a concentration as low as 0.002 molar.

[3] Experiments in which the accumulation of Br from unbuffered solutions and from solutions buffered with phosphate suggest that phosphate ions fall in this respect in the same category with SO_4 or NO_3 ions.

Evidently in these experiments, we have a very clear illustration of one anion influencing the intake and accumulation of another anion. These effects occur in very dilute solutions and with the exception of the higher concentrations of iodide, apparently do not involve injury; therefore it is doubtful whether we should refer to them as phenomena of antagonism. Naturally this depends entirely on the definition one may desire to apply to

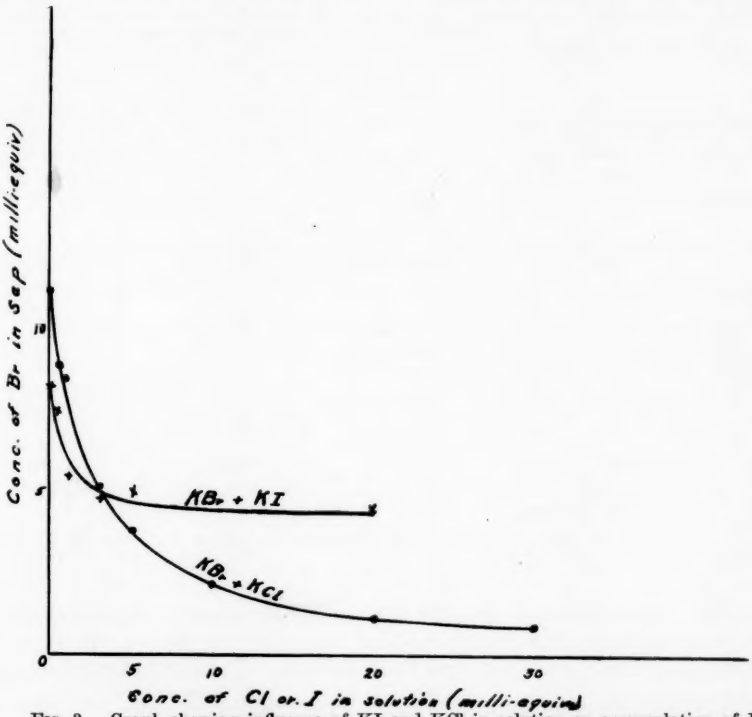


FIG. 3. Graph showing influence of KI and KCl in solution on accumulation of Br in cell sap. Exp. 1, 0.001 M. KBr solutions to which were added KI as indicated. Five days' duration; diffuse daylight plus continuous artificial illumination from two 300-watt lamps. Temperature, 20°–25° C. Initial pH, 5.6–5.8. Final pH, 5.8–6.2. Exp. 2, 0.001 M. KBr solutions to which were added KCl as indicated. Nine days' duration; illumination as in exp. 1; temperature 20°–25° C.; pH values similar to those of exp. 1.

the term "antagonism." When the cells were exposed to certain solutions of iodides during a period of ten days or more, a definite toxicity was observed in several of the experiments, whether caused directly by the accumulation of I ions, or by the formation of molecular iodine, subsequently. The presence of Br ions delayed or inhibited such toxicity. Pos-

sibly this might be considered as an example of antagonism occurring in dilute solution and becoming manifest only after a considerable interval of time.

TABLE I

EFFECT OF OTHER ANIONS ON ACCUMULATION OF Br IONS IN CELL SAP (Br PRESENT IN ALL SOLUTIONS AS 0.005 M. KBr)

CONDITIONS OF EXPOSURE	CONCENTRATION OF ADDED SALT (MOLAL)	CONCENTRATION OF Br IN SAP (MILLIEQUIVALENT)
	Experiment I	
Diffuse daylight, 10 day period, phosphate buffer solution* used. Room temperature.	none	15.2
	K_2SO_4 0.005	19.3
	KCl 0.005	7.3
	KI 0.005	8.2
	Experiment II	
Diffuse daylight, 12 day period, phosphate buffer solutions* used. Initial pH 5.0, final 5.8–5.9. Room temperature.	none	32.0
	K_2SO_4 0.005	35.0
	KNO_3 0.005	31.8
	KCl 0.005	13.8
	KI 0.005	15.5

* General buffer solution
0.002 M. $Ca(H_2PO_4)_2$
0.005 M. KH_2PO_4
Initial PH of such solutions usually between 5.0 and 5.4 and final pH between 5.6 and 6.0.

The results obtained with Br and I give an opportunity for relating potential rapidity of accumulation and reciprocal ion effects. It appears that anions which themselves are incapable of rapid accumulation are also incapable of having a marked influence on the accumulation of other anions. For example, sulphate ions, which are capable of only slow accumulation, do not retard the accumulation of Br appreciably. While it is possible for I ions to accumulate in the cell sap in considerable concentration, several experiments indicate that the accumulation of Br ions may be decreased through the presence of I ions in the solution, without the latter ions being accumulated in the sap in an amount equivalent to the decrease of Br ions. Obviously there exists a reciprocal relation between Br and I ions, so that the sum of the equivalents of halogens accumulated from the single salt solutions may be much larger than the corresponding value for the mixed solution. This means, of course, a mutual hindrance to penetration into

TABLE II

RELATIONS BETWEEN BR AND I IN ACCUMULATION

CONDITIONS OF EXPOSURE	BR IN SOLUTION (INITIAL) MILLI-EQUIV.	I IN SOLUTION (INITIAL) MILLI-EQUIV.	BR IN SAP MILLI-EQUIV.	I IN SAP MILLI-EQUIV.
Experiment I†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination for 6 days. Total period 10 days.	5.0	.....	58.6	.....
	.....	5.0	.....	19.9
	5.0	5.0	32.6	1.5
Experiment II†				
In light chamber.* 1,800 watts continuous illumination. Period 10 days.	5.0	.....	55.5	.....
	.....	5.0	.....	30.1
	5.0	5.0	41.5	6.8
Experiment III†				
Diffuse daylight and artificial illumination (2–300-watt lamps) 10 days, and 5 days in light chamber,* 3,000 watts. Continuous illumination throughout.	5.0	.....	61.7	.....
	5.0	5.0	46.6	4.0
Experiment IV†				
Diffuse daylight and artificial illumination (2–300-watt lamps) continuous illumination, 11-day period.	5.0	.....	22.0	.....
	.....	5.0	.....	6.0
	5.0	5.0	24.7	3.1

* A glass chamber 56 in. × 26 in. with lights evenly distributed outside chamber about 1 ft. from glass.

† Experiments I and II used phosphate buffer solution (table I) pH values 5.4–6.0. Experiments III and IV no buffer used. Similar pH values. Temperature in experiments not controlled, but varied between 20–25° C. Comparisons to be made within each individual experiment. In experiment II one lot of cells immersed in KI solution were too severely injured to be used. In experiment III nearly all cells in KI solution were killed. Cells in KI plus KBr solution in good condition.

the sap occurring somewhere in the protoplasmic layer. Apparently anions which do not have the potentiality of rapid accumulation in the sap are also incapable of penetrating the protoplasm to such an extent as to interfere with the accumulation of other ions. In comparing Br and I it will be observed that Br had a much greater relative effect on the accumulation

of I than I had on the accumulation of Br, and that Br could be accumulated by the cell more rapidly than I. If we can compare these results with the well known antagonistic effects between cations it is clear that anions may "antagonize" each other in an equally significant way, even in very dilute solutions.

We shall discuss next the possible influence of cations on the accumulation of anions. To test this possibility, experiments were made with solutions of various bromides and the accumulation of Br ions in the cell sap determined. It is clear from fig. 4 that the type of cation employed did

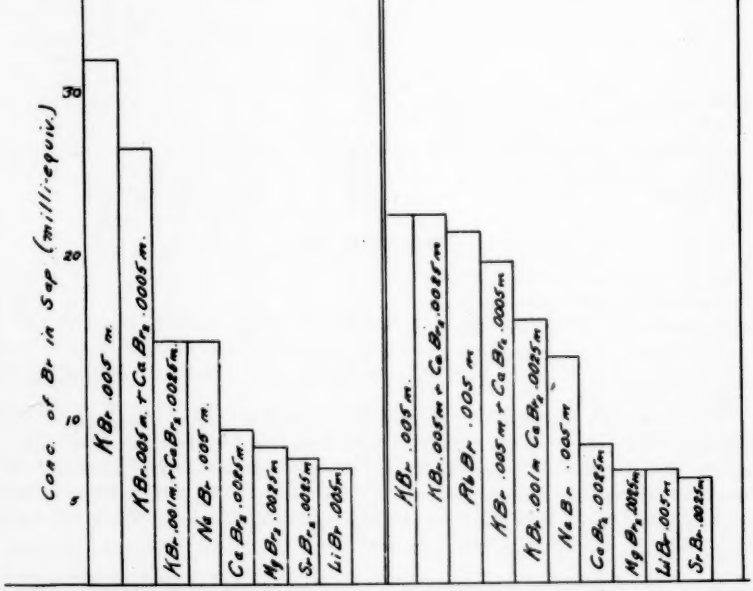


FIG. 4. Graphic representation of accumulation of Br in cell sap from solutions of various bromides. Exp. 1, three days' duration; daylight supplemented by artificial illumination at night from two 300-watt lamps; room temperature (20°–25° C.). Exp. 2, two days' duration; daylight plus continuous artificial illumination from two 300-watt lamps; room temperature (20°–25° C.). Initial pH of all solutions similar (5.6–5.8).

have a marked influence on the concentration of Br attained in the sap after a given exposure to the bromide solutions. The cations fall in three general groups, K and Rb; Na; Ca, Mg, Sr, Li, in the order of decreasing Br accumulation. These general relations were entirely consistent in two experiments, using different periods of time and conditions of exposure. Duplicate determinations of Br were made on each sample of sap with excellent agreement.

In several instances mixtures of KBr and $CaBr_2$ were used. In these cases (fig. 4) K had the dominant influence so that considerable excess of Ca had to be present in order to lower markedly the accumulation of Br from KBr solutions. These relations are not entirely similar to those ordinarily met with in experiments on antagonism, in which very small proportions of Ca may cause great changes in the physiological properties of a solution. In the present study the rate of accumulation of the anion was conditioned primarily by the presence in suitable concentration of a cation capable of ready penetration and accumulation rather than upon alterations in the protoplasm resulting from different proportions of mono- and di-valent ions. Probably a distinction should be made between cation-anion relations and those just discussed with reference to anions. The cation-anion relation may be electrostatic in nature and occur at outer surfaces.

In discussing the cation-anion relations it is suggestive to recall an experiment in which *Nitella* cells were allowed to accumulate Cl from a KCl solution to a concentration significantly greater than that normally existing in the cell sap. When these cells containing the increased amount of Cl were placed in a KBr solution they did not accumulate Br nearly so rapidly as similar cells taken from tap water. The Br which was accumulated by the former cells was accounted for by a nearly equivalent displacement of Cl from the sap, whereas the cells with the normal Cl content accumulated Br as a result both of exchange of Br for Cl and of simultaneous intake of K and Br. With the information now at hand these results can be explained on the assumption that the accumulation of Cl in the preliminary period was accompanied by an accumulation of K to a point approaching equilibrium, so that in the subsequent period of exposure to a KBr solution the cells could not readily accumulate an additional amount of K, thus restricting the Br accumulation to that represented by an exchange of Cl and Br. If this reasoning be correct we have another interesting example of the influence of the accumulation of the cation on the accumulation of the anion.

Since cation-anion and anion-anion interrelations exist it might be anticipated that one cation could influence the accumulation of another cation in dilute solutions, and we believe this to be true. However, it is not prac-

ticable at present to obtain satisfactory quantitative evidence with *Nitella* cells. The most rapidly accumulated cations, K and Rb, are exceedingly difficult to separate by any analytical method, while Cs is probably toxic. In experiments with plants of the agricultural type, in which accumulation of most ions proceeds sufficiently rapidly, it is possible to obtain very definite evidence of inter-ionic relations between cations in dilute solutions of the ordinary type.

It does not follow from the foregoing statements that large accumulations of Br ions may not take place from solutions of salts, the cations of which accumulate very slowly, for the contrary is true. Under such circumstances, Br ions penetrate into the cell chiefly in exchange for Cl ions, and the latter enter the outside solution, as has been pointed out in a previous paper. (When Br is accumulated from KBr solutions, simultaneous accumulation of K and Br is involved as well as exchange between Cl and Br.) Furthermore there is the possibility that ions may enter the cell to some slight extent in conjunction with H or OH ions, although the available data do not indicate that this method of accumulation is of primary importance in the experiments under discussion.

The influence of hydrogen-ion concentration on the accumulation of Br ions

The rôle of hydrogen-ion concentration in the accumulation of other ions is undoubtedly a very complicated one. When we used masses of very small young cells and examined the culture solution, instead of the cells themselves, we found that the removal of Br or Cl ions was much more pronounced from an acid solution (pH 5–6) than from an alkaline one (pH 8–9). A similar effect was shown for the entrance of NO_3 into large cells by direct examination of the cell sap of individual cells. We have not been able to show, however, equally marked effects of hydrogen-ion concentration on the accumulation of Br by large cells, although the indications are that a pH of 6–7 is most favorable to the accumulation. It may be noted that M. M. Brooks (1) in studying the penetration of methylene blue into *Vallonia* cells finds that the rate and not the equilibrium value is influenced by reaction. Our results do not necessarily give an accurate idea of rates.

It is certain that large accumulations of Br ions may take place at any of the pH values experimented with, namely, pH 5–8 (table III). In passing, attention is called to the observation that Br ions may become much more concentrated in the cell sap than in the culture solution even when the pH value of the latter is the same as that of the cell sap, so that no gradient of H or OH ions is necessarily required for the process of accumulation. This fact may be of interest in connection with hypotheses concern-

TABLE III

EFFECT OF HYDROGEN-ION CONCENTRATION ON ACCUMULATION OF Br

CONDITIONS OF EXPERIMENT	INITIAL pH	FINAL pH	CONCENTRATION OF Br IN SAP MILLIEQUIV.
Experiment I*			
Diffuse daylight plus continuous artificial illumination (2–300-watt lamps), 5-day period. Room temperature.	5.4	5.6	20.7
	6.4	6.8	31.7
	8.2	7.6	20.6
Experiment II*			
Light chamber, 3,000 watts continuous illumination, 3-day period. Temperature 20–25° C.	5.4	....	30.6
	6.4	....	35.0
	7.6	....	27.9
Experiment III*			
Diffuse daylight, 3-day period. Room temperature.	5.5	5.9	7.4
	6.4	6.6	17.7
	7.4	7.4	16.9
	8.6	7.4	12.7
Experiment IV*			
Diffuse daylight, 5-day period. Room temperature.	5.2	6.0	15.6
	6.0	6.6	31.0
	7.0	7.2	34.4
	8.0	7.9	27.8
	8.3	7.9	19.5
Experiment V*			
Continuous artificial illumination (2–300-watt lamps), 3-day period. Average temperature, 24° C.	4.9	5.5	19.3
	6.0	6.0	24.4
	6.9	6.9	26.4
	7.8	7.8	30.4

* KH_2PO_4 plus NaOH used to make buffer solution. Experiments I, II, III, KH_2PO_4 0.001 M. Experiments IV, V, K_2PO_4 0.0025 M. KBr 0.005 M. present in all solutions.

ing the mechanism of accumulation. It is also worthy of comment that the accumulation of both cations and anions takes place on the alkaline side of the average isoelectric point of the proteins present in the cell, as determined by PEARSALL (**8**). There does not seem to be available any explana-

tion of ion accumulation on a simple basis of ion protein combinations. It would be difficult to assume that individual proteins are available of such widely different isoelectric points as to permit the simultaneous formation of both cation and anion compounds at the pH values involved.[4]

General discussion

It will be remarked that we have explained all of our data in terms of ions. We are, of course, aware that the hypothesis has been advanced that only undissociated molecules are able to penetrate into living cells. Certain experiments with weak acids and with dyes have been made which lend support to this hypothesis (**6, 7**). (Some work by M. M. BROOKS (**1**) on dyes tends rather to an interpretation in terms of ions.) Concerning these data we do not know to what extent they bear on the results we have presented. As we have suggested previously, in the systems containing the weak acids employed, gaseous components were present and it is not certain that they can be disregarded. In the solutions with which we have experimented, the salts were possibly completely dissociated according to present theories, and in the sap the salts also must have existed primarily in dissociated form for reasons discussed elsewhere. It would require very complicated assumptions to explain our results on any other than an ionic basis. For example, we have to consider the unequal accumulation of the ions of a salt such as $CaBr_2$, the Br accumulation in this case being accompanied by a marked loss of Cl from the cell. An explanation is required also for the very different effects of different anions on the accumulation of Br. In fact, all of the observations seem to be most easily understood by assuming that ionic processes are involved. It is true, however, that the actual mechanism of accumulation is as yet unsolved, so that it would be unwarranted to make positive statements, especially as the physical chemistry of even simple ionic systems appears to be in a stage of further development.

Although the data we have discussed in this paper are confined to one algal organism, we feel that the general relations which have been shown to exist between the cell sap and the culture medium apply to a wide range of plants. Indeed our experiments on agricultural plants are consistent with this statement. With regard to the comparative rates of accumulation of specific ions it would seem that this question is not wholly one of ionic properties, but involves the type of plant metabolism as well. For example, many agricultural plants are capable of very rapid accumulation of nitrate,

[4] Note, however, recent article by G. E. BRIGGS and A. H. K. PETRIE, Biochemical Jour., Vol. XXII, No. 4, pp. 1071–1082 (1928) also J. Davidson, Jour. Agr. Res. Vol. 35, No. 4, pp. 335–346 (1927).

but this does not seem to be true of *Nitella* cells. If we consider the cation relations, as they are reflected by the present experiments, the physiological order would be consistent in a general way with the mobilities of the ions, but with the anions the matter is in doubt. A complex ion like NO_3 or SO_4, capable also of reduction, is very difficult to place on a purely electrochemical basis.[5] Comparing the results on other plant cells, obtained by STILES and KIDD (**10**) and by several other investigators, there is a general agreement as to the order of the ions, with the exception of the NO_3 ion, as already mentioned. Recently, COOPER and WILSON (**2**) have suggested that the electromotive force is the most important characteristic of the ion to be considered, but the details of their researches are as yet unavailable.

Summary

1. Further experiments on *Nitella* cells are reported pertaining to the accumulation of halogens in the cell sap. Analyses were made of composite samples of sap obtained from individual cells. Consideration was given to the maintenance of conditions of illumination essential to the process of accumulation.

2. The relation between Br ion concentrations in the culture medium and in the cell sap was of a logarithmic type. This is in agreement with results obtained on other plant cells using other electrolytes.

3. The accumulation of Br ions was significantly retarded by Cl or I ions also present in the culture medium, but not by SO_4 NO_3 or PO_4 ions. These and other ionic effects discussed occurred in very dilute solutions. Consideration is given to the relation between the retarding effects of one ion on another and potentiality of accumulation in the sap.

4. The accumulation of Br ions was definitely influenced by the nature of the cation, being most rapid when solutions of KBr or RbBr were used, and least rapid with solutions of LiBr, $CaBr_2$, $SrBr_2$ and $MgBr_2$.

5. With regard to the effects of hydrogen-ion concentration, marked accumulation of Br ions took place at reactions varying between pH 5 and 8.

6. Taking all the data into consideration it is found very difficult to explain them on any other than an ionic basis.

LABORATORY OF PLANT NUTRITION,
UNIVERSITY OF CALIFORNIA.

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THE PREPARATION OF CHLOROPHYLL*

F. M. Schertz

Introduction

This paper is published in response to repeated requests for information regarding the preparation of pure chlorophyll. It also shows the relative purity of the samples of chlorophyll which have been used as the basis of investigations conducted in this laboratory. It is hoped that the method here described will be helpful to those contemplating a study of this important pigment. Chlorophyll is so important that it is felt that every effort should be put forth by workers to obtain a product of undoubted purity. An effort has been made to present the details of the preparation so that the difficult parts may be mastered by most investigators after a little preliminary work on their part.

Material used

The choice of material to be used in the preparation of chlorophyll is very important for in the leaves of many species of plants chlorophyll decomposes during the process of drying. This is true of leaves of the following plants: alfalfa, spinach, cowpea, and many others. In fact very few leaves will dry entirely satisfactorily. Consequently, in order to save much valuable time and material it is best to use the leaves of only such species as have proven entirely satisfactory. Leaves of *Urtica dioica* have been shown by experience to be best, while those of *Urticastrum divaricata* are very good.

The leaves for our preparation were collected during the active growing season (April or May) and were then spread out on screens to dry, away from direct sunlight. A temperature of 30–40° C. was used for drying. Twenty-four hours are usually sufficient. Electric fans and hot plates were used to hasten the drying process. As soon as the leaves were dry enough to crumble easily in the hands, they were ground finely in a pebble mill. The material should not be ground too finely otherwise the extraction will be more difficult. The method of extraction and purification as given here is the method which has been found to remove the impurities most satisfactorily. All of the operations in the preparation of chlorophyll should be carried out as quickly as possible.

* Soil-Fertility Investigations, U. S. Department of Agriculture, Washington, D. C.

Method of extraction and purification

Two moistened filter-papers are placed in a 25-cm. suction funnel (this size of funnel is used because the layer of meal should not be more than 4–5 cm. thick), and sucked down firmly. One kg. of the leaf meal is loosely placed into the funnel and 2 liters of 80 per cent. acetone (by volume) are poured on and allowed to sink into the meal till some of the extract runs from the funnel below. Suction is applied and the extract is sucked off into a filter-flask. Suction at no time should make the acetone extract boil or the filtration will be hindered. More acetone (1 liter of 80 per cent.) is added and sucked off. This is repeated till all of the green pigment is extracted from the leaf meal. About 3–6 liters of the 80 per cent. acetone are required for the extraction. Finally, the decolorized leaf meal is sucked dry to remove all of the acetone extract from the leaf powder.

The vivid green extract is transferred into 1.5 l. of petroleum ether (B. P. 30–70° C.) in a 6-liter separatory funnel (a). The petroleum ether will take up the four chloroplast pigments and a weakly yellowish green aqueous acetone layer will separate below. This aqueous acetone layer is run in a thin stream through a 4-l. separatory funnel (b) which contains 1 l. of petroleum ether. The petroleum ether in this funnel will remove practically all of the chloroplast pigments. The aqueous acetone from which the chloroplast pigments have been separated is poured into a 6-liter separatory funnel (c) which contains 0.5 l. of petroleum ether. This last separatory funnel of petroleum ether is to break emulsions which will form in the process described in this paper and to remove any traces of chlorophyll which may have passed through the other funnels.

The petroleum ether in the three funnels is now washed with a liter of 80 per cent. acetone which removes impurities and takes away none of the chlorophyll. The washing is carried out by allowing the acetone to run in a fine stream through the petroleum ether solutions. The petroleum ether by taking up acetone has increased considerably in volume. The acetone is removed by allowing a liter of distilled water to flow into *a* and then on down through *b* from a liter separatory funnel (*d*) above *a*. By the above method, much of the accompanying substances are removed, the greater per cent. of the acetone is separated and emulsions are prevented.

The solutions are never shaken at any time throughout this procedure, for emulsions once formed are difficult to break and this method prevents their formation to any great extent. All of the wash liquors from *b* are passed through funnel *c* from which they are run off. A large per cent. of the acetone may be recovered from the aqueous acetone by distillation, and used again.

The acetone is not quantitatively washed from the petroleum ether solutions, for the chlorophylls and xanthophyll would precipitate, and the purification would be made much more difficult; for then the xanthophyll could not be separated from the petroleum ether by the methyl alcohol washings.

The xanthophyll is now separated from the chlorophylls and carotin by allowing 4 liters or more of 80 per cent. (by volume) of methyl alcohol to run down in a fine stream through the petroleum ether in the separatory funnels. The last liter of methyl alcohol may be cautiously shaken with the petroleum ether solution of chlorophyll. The methyl alcoholic extract of xanthophyll is run out of each separatory funnel into the next as rapidly as it separates from the petroleum ether layer, for in this way more of the xanthophyll will be removed from the petroleum ether layers and a purer chlorophyll product will result. The methyl alcoholic extracts may be worked up for xanthophyll by the methods described by WILLSTÄTTER and STOLL.

The last traces of methyl alcohol and acetone are now removed by allowing a fine stream of water to run from separatory funnel *d* down through the petroleum ether solutions. In all, 8 to 12 liters of distilled water are required for this washing. The petroleum ether gradually loses its fluorescence, becomes turbid and the chlorophylls precipitate. Toward the end of the washing, emulsions will form but these may be broken by dissolving salt (NaCl) in the wash water from funnel *b* and then the saline solution is poured into separatory funnel *c*, where most of the emulsion will disappear. If the emulsion still persists it may be broken by running off the aqueous layer from funnel *c* and adding more salt. The saturated solution is returned to funnel *c* and this process is repeated till most of the water is removed from the petroleum ether layer.

The petroleum ether suspension of chlorophyll is now shaken with about 250 gm. of anhydrous Na_2SO_4 which removes the last traces of water and makes the solution filterable. The petroleum ether and accompanying impurities are now separated from the chlorophyll by filtering through a layer of talc on a 25 cm. suction funnel. The layer of chlorophyll which forms upon the talc is constantly broken by stirring with a nickel spatula, otherwise filtration would proceed very slowly. The petroleum ether filtrate obtained contains very little chlorophyll, practically all of the carotin and only a very little of the xanthophyll. From this filtrate carotin may be obtained. The suction applied in this filtration should be very moderate else the filtration will be retarded. Finally, wash the mass of chlorophyll and talc with 500 cc. or more of petroleum ether. Then, apply strong suction to remove as much of the petroleum ether as possible.

A. The acetone method of purification

The chlorophyll-containing talc is removed from the suction funnel and placed in a beaker where it is stirred for a short time with pure acetone (500 cc.). The acetone solution of chlorophyll is now filtered from the mass by placing the whole upon a suction funnel (15 cm. in diameter) and washing the talc with acetone till all of the chlorophyll is removed.

The acetone solution of chlorophyll is poured into 1 l. of petroleum ether in separatory funnel *a* and 500 cc. of petroleum ether is added to each of the funnels *b* and *c*. Water is now added to the chlorophyll solution until the acetone layer separates. This layer is run through the petroleum ether in *b* and *c*, after which the acetone may be recovered. The acetone is now all washed from the petroleum ether solutions by washing as above with 8–12 liters of distilled water. The chlorophyll precipitates and is obtained by filtering the dried (with anhydrous Na_2SO_4) petroleum ether solution through talc upon a suction funnel (15 cm. in diameter). The precipitated chlorophyll is washed with 0.5 to 1 l. of petroleum ether. The petroleum ether mother liquor here should run off slightly yellowish at first and finally only faintly green.

The chlorophyll-containing talc is now freed of the petroleum ether by allowing the suction to continue for a few minutes. The material may be allowed to stand over night in this condition. This purification has removed traces of carotin and xanthophyll. The chlorophyll is now purified by the petroleum ether method which removes practically all of the impurities.

B. The petroleum ether method of purification

The chlorophyll-containing talc is now removed from the funnel and placed in a beaker where it is stirred for a short time with alcohol-free ether (500 cc.).[1]

As soon as the chlorophyll has gone into solution it is filtered through a thin layer of talc on a separatory funnel (15 cm.) which is just large enough to hold the mass of material. The talc is washed with ether till all of the chlorophyll is removed.

The ether (1 l.) is now evaporated from the chlorophyll solution until a sirupy mass remains. The evaporation is carried out by placing the container in a hot water bath. The level of the hot water is always kept below the level of the ether in the container. When the volume of the chlorophyll solution is reduced to about 100 cc. the ether is then removed by reduced pressure till the mass is sirupy. About a liter of petroleum ether is added a little at a time and the flask is shaken violently till the chlorophyll precipitates.

[1] The ether used here is prepared by washing U.S.P. ether with water several times and then distilling over $CaCl_2$.

The precipitated chlorophyll is now removed from the mother liquor by filtering upon talc and is then washed with about 500 cc. of petroleum ether.

It is extracted again with 500 cc. of purified ether and the solution is concentrated at 30–40° C. to a sirupy mass which is then dried in a beaker in a vacuum desiccator. When dry the steel blue shiny mass of chlorophyll may be easily pulverized and weighed.

TABLE I

YIELD OF CHLOROPHYLL ($\alpha + \beta$) OBTAINED FROM LEAVES FROM DIFFERENT SOURCES

SOURCE	METHOD OF PURIFICATION	YIELD IN GM. PER KGM. OF DRY LEAVES
Minnesota, 1924	Ether	2.0
"	Ether	1.4
Missouri, 1924	Ether	6.9
"	Ether	7.1
"	Ether	8.3
"	Ether	8.0
"	Ether	7.9
"	Ether	8.4
"	Ether	8.2
"	Acetone	7.1
Washington, D. C., 1924	Acetone	4.5
"	Acetone	4.3
"	Acetone	3.8
"	Acetone	5.6

Purification of chlorophyll by the methods described here has given the writer his purest product. Consequently, it is submitted as a method of preparing very pure chlorophyll.

Results of extraction by the above methods

Samples of stinging nettle leaves were obtained from Minnesota for extraction and the amount of chlorophyll obtained per kg. from them is compared with that from stinging nettle leaves collected at Washington, D. C. Chlorophyll was also extracted from the leaves of wood nettles from Missouri.[2]

The amount of chlorophyll obtained per kg. of the dried leaves is recorded in table I.

The yields of chlorophyll as given in this table were not all obtained by the method which has just been described above. The first nine were pre-

[2] The author is greatly indebted to Dr. CARL G. DEUBER, formerly of the University of Missouri, now of Yale University, for collecting and drying these leaves.

pared by the method given by WILLSTÄTTER and STOLL and the remainder were prepared by the same method except that they were purified by the acetone method instead of being precipitated by petroleum ether as given by WILLSTÄTTER and STOLL in their monograph on chlorophyll.

The quantity of chlorophyll per kilogram from the leaves from Minnesota was the lowest of all and also the pigment which was obtained was off color when dissolved in ether. The wood nettle leaves obtained from Missouri gave the best results and the chlorophyll obtained was the purest.

Using 0.0513 grams of each sample of chlorophyll[3] (or 0.10 gm. per liter) tests were made for total yellow pigments, and with the colorimeter also to determine which samples contained the least impurities. Those containing the least impurities when saponified gave the lowest readings on the colorimeter. The carotinoids were separated from the chlorophyllins and the results are recorded in table II.

The results shown in table II are for only the samples which were used in the final work on chlorophyll. Many other samples not described or used in this work showed that changes had taken place in the chlorophyll during its extraction and purification, or that the original leaf material was not of the best grade. Samples which did not show a pure green color when dissolved in ether or which failed to give a good phase test were not used.

In the measurements of chlorophyll in columns 3 and 7 of table II, the readings are in millimeters, and the comparisons are made with the following combination of Lovibond slides, nos. 3, 4, and 5 blue, plus 10 and 20 yellow, using a Duboscq colorimeter. The concentration of chlorophyll in the solutions measured is 0.1000 gm. per liter.

The carotinoids, shown in columns 4 and 8 of table II, were estimated colorimetrically as carotin. The amounts given were the amounts found in each 0.05 gm. sample of chlorophyll.[4]

The readings for samples no. 4 and no. 6 show that most of the carotinoids were removed by the acetone method of preparation, as can be seen by the figures in column 3, table II. All of the samples given in column 3 were then purified by the acetone method as given in this paper. Examination of the figures in the last four columns of table II shows that the method was not only valuable in removing the carotinoids but the purity of the chlorophyll samples was greatly improved. (*Cf.* columns 3 and 7 for chlorophyllin content.)

[3] The samples of chlorophyll used here were dried in a vacuum desiccator for two weeks.

[4] SCHERTZ, F. M. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. 26: 383–400. 1923.

TABLE II

PURITY OF CHLOROPHYLL SAMPLES AS SHOWN BY TESTS OF THEIR CAROTINOID AND CHLOROPHYLL CONTENT, AND THE RESULTS OF PURIFICATION BY THE ACETONE METHOD

SAMPLE NUMBER	CHLOROPHYLL YIELD PER KILOGRAM OF DRY LEAVES	CHLOROPHYLLIN CONTENT (COLORIMETER)	CAROTINOID CONTENT	WEIGHT OF CHLOROPHYLL AFTER ACETONE PURIFICATION	LOSS IN PURIFYING	CHLOROPHYLLIN AFTER ACETONE TREATMENT (COLORIMETER)	CAROTINOIDS AFTER ACETONE TREATMENT
	gm.	mm.	mg.	gm.	Per cent.	mm.	mg.
1	8.9	24.9	0.73	5.6	30.0	17.7	0.50
2	8.4	16.5	0.73	6.6	21.0	16.1	0.43
3	8.2	23.2	0.77	6.2	24.0	17.4	0.46
4*	4.3	27.5	0.10	2.9	32.0	16.8	less than 0.10
5	6.9	23.1	0.30	5.7	17.0	15.0	less than 0.10
6*	7.1	24.8	0.10	5.0	29.0	17.8	less than 0.10

* Samples 4 and 6 were prepared by the acetone method while the others were obtained by the ether method.

Further evidence may be submitted in favor of incorporating the acetone method of purification with the method for preparing fairly pure chlorophyll. In all, five samples of chlorophyll were obtained by substituting the acetone method for the ether purification as used by WILLSTÄTTER and STOLL. One of the samples of chlorophyll, when extracted and prepared by the acetone method contained 0.20 mg. of carotinoids while the other four samples contained less than 0.10 mg. per 0.0513 gm. sample of chlorophyll. The average result for fifteen samples obtained by the ether method of WILLSTÄTTER and STOLL was 0.81 mg. of carotinoids per 0.0513 gm. of chlorophyll. The method as modified, then, should be far superior to the method given by WILLSTÄTTER, because not only will the carotinoid content of the samples obtained be less, but the chlorophyll content will be much greater, as shown by the figures in table II.

The samples used here would have been further purified if any method had been available whereby a purer sample could be detected. The error in colorimetric estimation was too great to make any further purification worth while and spectrophotometric methods used with the yellow pigments are not applicable to a solution of mixed pigments. Consequently, these six samples after further testing were regarded as pure chlorophyll, though it is highly probable that they contain a small percentage of impurities.

Before any quantitative experiments could be carried on with the chlorophyll prepared it was necessary to know the loss in weight on heating to 100° C. so that the correct amount of chlorophyll could be weighed out for any given experiment.

Table III shows the weight of the samples of chlorophyll before and after drying in an electric oven over night at 100° C. after the samples had been previously dried in a vacuum desiccator for two weeks. The loss in the samples was generally 0.0007 gm. consequently, 0.0507 grams, which represents 0.0500 gm. of dried chlorophyll, was used in each experiment.[5] In all of the acetone purification experiments in table II, 0.0507 gm. of chlorophyll was used, instead of 0.0513 gm. which was used by WILLSTÄTTER.

The first six samples in table III are those already described in this paper. Samples 7 and 8 in table III were prepared after the ash content of the first six were known. These two samples were prepared by the method described in this paper. The ash content was then determined, the data being given in the last column of table III. These two samples were then again purified by dissolving in ether and precipitating by the addition of petroleum ether. The ash content was then found to be exactly the same

[5] Chlorophyll cannot be dried at 100° C. and then used, for the heat alters the chlorophyll molecule. Chlorophyll after drying is brownish green instead of a pure green when dissolved in ether. If a dried sample is saponified the result is a dirty brownish green chlorophyllin instead of a clear bright green.

TABLE III

LOSS IN WEIGHT BY DRYING CHLOROPHYLL AT 100° C. AFTER DRYING IN A VACUUM DESICCATOR FOR TWO WEEKS OR MORE, AND THE ASH CONTENT OF CHLOROPHYLL PREPARATIONS

SAMPLE NUMBER	WEIGHT BEFORE DRYING	WEIGHT AFTER DRYING	LOSS IN DRYING	ASH CONTENT OF CHLOROPHYLL
	gm.	gm.	gm.	Per cent.
1	0.0513	0.0506	0.0007	3.9
2	0.0519	0.0513	0.0007	4.2
3	0.0520	0.0513	0.0007	3.6
4	0.0514	0.0507	0.0007	3.4
5	0.0520	0.0513	0.0007	4.5
6	0.0507	0.0495	0.0012	4.1
7	.....	.....	.....	4.1
8	.....	.....	.....	4.0

as that already determined. Hence, no further attempts to improve the purity of the chlorophyll were made.

Only one of the samples reported in table III gave the theoretical amount (4.5 per cent.) of ash and this sample was not purified as much as some of the others. No explanation is offered as to why the ash content varies in the samples. It might be suggested that the magnesium of the chlorophyll had been replaced by hydrogen but this was not true, for each of the samples of chlorophyll was carefully tested in the spectroscope for bands due to pheophytin. No trace of pheophytin was found in any of the samples. This test is easy to make for the spectroscopical bands of chlorophyll and of pheophytin are quite different.

The ash of two of the samples were tested for their MgO content. The ash from sample no. 1 was found to yield 100 per cent. MgO while that from no. 5 was found to be 92 per cent. MgO.[6] No other tests to determine the MgO content of the samples were made.

The ash content of samples of chlorophyll prepared by WILLSTÄTTER and his students will now be considered. WILLSTÄTTER and ISLER[7] prepared pure chlorophyll α and β. The amount of ash (MgO) found for the samples of chlorophyll α was 4.3, 4.6, and 4.2 per cent. and for chlorophyll β it was 4.1, 4.4, 4.4, and 4.2 per cent. WILLSTÄTTER and HUG[8] give only 3 results for preparations of pure chlorophyll. They are 4.5, 4.6 and 4.9 per cent.

[6] The author is indebted to R. B. DEEMER of this laboratory for these determinations.

[7] WILLSTÄTTER, R., and ISLER, MAX. Untersuchungen über Chlorophyll. XX. Über die zwei Komponenten des Chlorophylls. Liebig's Annalen. **390**: 269–339. 1912.

[8] WILLSTÄTTER, R., and HUG, ERNST. Untersuchungen über Chlorophyll. XV. Isolierung des Chlorophylls. Liebig's Annalen. **380**: 177–211. 1911.

Their preliminary results were 3.1, 3.2, 3.9, 3.7, and 3.6 per cent. The pure chlorophyll which WILLSTÄTTER and HUG prepared was obtained by a method which was essentially different than that described in this paper. The leaf meal was given a preliminary washing with benzol and with petroleum ether. The chlorophyll was then extracted with ethyl alcohol, transferred to petroleum ether and then purified. Results of analysis for ash are not given in WILLSTÄTTER and STOLL's monograph on chlorophyll, in which they describe the method which has been modified and described in this paper. Why the results given by WILLSTÄTTER and HUG are higher than the theoretical and why the results given in this paper are lower than the theoretical result, 4.5 per cent., is as yet unexplained.

Tests used in determining purity

PHYTOL CONTENT

Instead of directly determining the phytol content of the chlorophyll, its purity was determined by extracting an ethereal solution of the pigment with 22 per cent. HCl. If the chlorophyll has been altered by the splitting off of the phytol this fact will be indicated because any chlorophyllide present will dissolve in the 22 per cent. HCl. This test is very sensitive.

None of the ethereal solutions of chlorophyll gave any color when extracted with 22 per cent. HCl, hence none of the phytol was split off. If phytol were split off the MgO content would have been too high.

YELLOW PIGMENTS

In the results given in this paper it is seen that none of the chlorophyll preparations was wholly free from yellow pigments, nor has the author succeeded yet in preparing one which is absolutely free from carotinoids.

Most of the preparations here described contained much less than 1 per cent. of the carotinoids; the carotinoid present is xanthophyll.

THE PHASE TEST

The brown phase appeared on saponification with methyl alcoholic potash. Allomerized chlorophyll would not give this test and the solution remains brown with a mixture of pure and allomerized chlorophyll.

THE SPECTRUM

An analysis of the spectrum of the preparations was made to see whether any of the groups in the chlorophyll molecule had been changed in the process of preparation. This observation is far more sensitive in detecting impurities in the sample than would be a chemical analysis of the chlorophyll. If chlorophyll is altered by the action of acid, the presence of pheo-

phytin is observed in the spectrum, for then two absorption bands appear; one before the Fraunhofer line E and the other between the lines E and F. No such pheophytin absorption bands were found in the spectra of these preparations. The absence of these bands is evidence of purity of the samples.

The magnesium complex of all the preparations was found to be unaltered.

Color tests

In order to tell which preparations of chlorophyll were good, their ether extracts were compared as to color. Several samples were compared at one time and only those showing a pure green color were considered good. Traces of allomerization could be easily detected in this manner.

Allomerization was further tested for by saponifying these ethereal solutions of chlorophyll. Good chlorophyll showed a pure, clear green while allomerized chlorophyll was of a dirty green color, after the saponification.

Summary

1. A method is described for the extraction and purification of chlorophyll ($\alpha + \beta$).

2. From 7–8 grams of chlorophyll may be obtained from 1 kg. of nettle leaves.

3. Purification of chlorophyll by the acetone method described is recommended because by its use most of the carotinoids are removed and the chlorophyll was found to be much purer. The method as outlined is recommended for obtaining very pure chlorophyll. If large yields of chlorophyll, not so pure, are desired, then the method may be easily modified to meet the needs of the worker.

4. 0.0500 gram samples lost about 0.0007 gm. on drying at 100° C.

5. The chlorophyll preparations described here yielded on an average 3.98 per cent. of ash. Two samples of the ash were analyzed and found to contain 92 and 100 per cent. MgO.

6. The tests used in determining the purity of chlorophyll are described.

U. S. Department of Agriculture,
Washington, D. C.

A METHOD FOR THE DETERMINATION OF INORGANIC NITROGEN IN PLANT EXTRACTS[1]

A. C. SESSIONS AND J. W. SHIVE

(WITH ONE FIGURE)

Numerous methods have been used for the determination of nitrate nitrogen in plant tissue, but a review of the recent literature shows the Ulsch method (**1**), the Kjeldahl-Gunning-Arnold method to include nitrates (**1**), and the Devarda method (**9**), to be the three most widely used at the present time. All of these methods, however, have been severely criticized.

NIGHTINGALE, ROBBINS and SCHERMERHORN (**4**) called attention to the fact that nitrogen obtained by the Ulsch method includes not only nitrate nitrogen but also any amide nitrogen which may be present. GALLAGHER (**3**) criticizes the Ulsch method, for, as he states, in the reduction of nitrates by means of an acid reducing agent, the first step in the process appears to be the formation of nitrites. In the presence of acid, nitrous acid is formed, and this may react with amino groups which may be present, either in the form of amino acids or proteins, with the liberation of nitrogen. However, if heat is not applied too rapidly, the loss of gaseous nitrogen apparently does not occur (**4**) though the possibility of loss of nitrogen due to improper manipulation is a serious objection to the method, as well as the necessity of working with amide-free solutions. STROWD (**9**) concludes that the determination of nitrate nitrogen in plants by finding the difference between the Kjeldahl method to include nitrates and the Kjeldahl-Gunning-Arnold method is unsatisfactory, since appreciable amounts of nitrates are apparently reduced, even without the addition of reducing agents.

Many workers are now determining the nitrate nitrogen of plant extracts by the Devarda method. In this method, 0.4 to 1.0 gram of sodium hydroxide is added to each 100 cc. of the plant extract, and this alkaline solution is distilled with one to two grams of Devarda's alloy for one hour. The ammonia obtained from the reduction of the nitrates is absorbed in standard acid and titrated against standard alkali. This method was recommended by STROWD (**9**) in 1920, but BURRELL and PHILLIPS (**2**) could not obtain concurrent results when amide nitrogen was present in the extract. Recently RANKER (**8**) has shown that this method as ordinarily used gives values which in no way represent the nitrate nitrogen in biolog-

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

ical material. PHILLIPS (**5**) suggests that this method may be used successfully, provided that the ammonia and amide nitrogen is removed prior to the nitrate determination.

BURRELL and PHILLIPS (**2**) used the phenoldisulphonic acid method, but failed to obtain consistent results until they had cleared the extract, removed the acid-charred organic matter, and freed the solution from chlorides. Due to the complications which these processes introduce, this colorimetric method is little used.

PYNE (**7**) has recently developed two methods which appear to give reliable results. The first method is based upon the reducing power of titanous hydroxide in a cold alkaline solution; the second depends upon the reduction of nitrates by Devarda's alloy in the cold, the conversion of the ammonia formed into ammonium chloride, and distillation *in vacuo* at 45°–50° C. with ignited lime.

In the method to be described in the following pages, nitrates are reduced to ammonia by the use of cold eighth-normal alkali and finely ground Devarda's alloy. The ammonia thus formed is recovered by the Folin aspiration method.

The work was carried out in the Laboratory of Plant Physiology of the New Jersey Agricultural Experiment Station, and it is a pleasure to acknowledge indebtedness to Dr. G. T. NIGHTINGALE and to Dr. W. C. RUSSELL for many helpful suggestions and encouragement.

Materials

In the method here described, Merck's analytical reagents were used throughout. Sulphuric acid solution, 0.050 normal and 0.033 normal sodium hydroxide solution were used in all titrations. These were standardized against benzoic acid obtained from the United States Bureau of Standards (sample 39 C). Methyl red was used as an indicator, and all titrations were carried to the complete disappearance of any red tinge.

In obtaining the plant extracts, the fresh tissue was ground in a large mortar with nitrogen-free quartz sand. Water was added to the ground material and the whole was transferred to a large funnel over which had been placed an eighteen-inch square of lawn cloth. The extract was forced through the cloth by moderate wringing by hand. All nitrates could be removed from 150 grams of the plant materials here used by two thorough grindings and repeated extractions with two liters of water, as it was found that small portions of the extracted tissues oven-dried at 70° C. gave only a negative reaction with diphenylamine. The resulting extract was heated to boiling, 3 cc. of 10 per cent. acetic acid was added, and boiling was continued for 2 or 3 minutes. The coagulum formed was removed from the

solution by filtering through a Buchner funnel into which was placed a filter paper and nitrogen-free paper pulp. The coagulum-free filtrate was reduced in volume to 500 cc. by gentle boiling, and 50 cc. aliquots were taken for analysis. At no time was the extract thus prepared allowed to stand more than 12 hours before analyzing; usually the analyses were made immediately after extraction.

Apparatus and method

The absorption tubes and aspiration bottles used consist of large test tubes (2.5 × 25 cm.) and 300 cc. Florence flasks, respectively. Twelve of each constitute a battery, and are so arranged that the same current of air is drawn through the entire system. Figure 1 shows the essential features of the apparatus and its arrangement. Fifty cc. of the plant extract is placed in each of the Florence flasks and 2 cc. of paraffin oil is added.

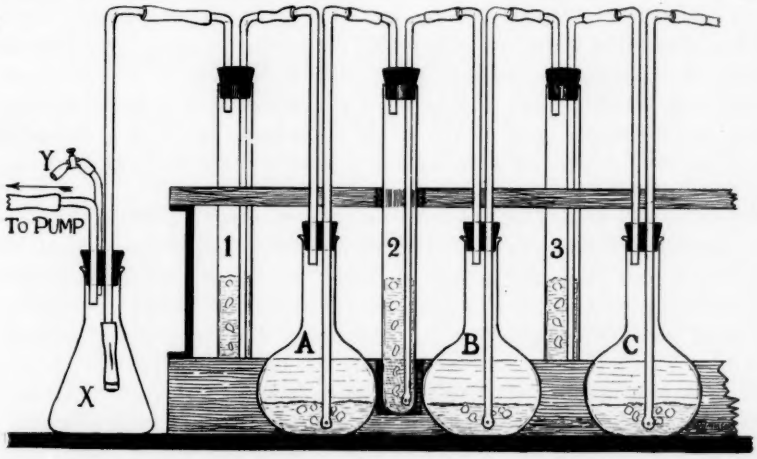

FIG. 1. Aspirating system for determining inorganic nitrogen in plant extracts. Description in text.

Beginning with flask A, 50 cc. of a saturated solution of sodium carbonate to which has been added 5 grams of sodium chloride, is now introduced, the stopper of the flask is immediately inserted, and connection is made by means of a short piece of rubber tubing between the outlet tube of the flask and the inlet of the aspiration tube 1, which contains the standard acid. A slow current of air is now started through the solutions of the first unit of the system, comprising flask A of the diagram of fig. 1 and its corresponding absorption tube 1. In a similar manner, the second unit of the system is now prepared and joined to the first by means of a short rubber tube con-

nection, as indicated in the diagram. Each unit is thus prepared and joined in the series in regular order until the battery is completed. Aspiration is allowed to proceed slowly at first, then gradually increased to the desired rate, and allowed to continue rather vigorously until all the ammonia is removed. In the work here reported, aspiration was always allowed to proceed during a period of not less than twelve hours.

Aspiration is accomplished by the use of an ordinary filter pump attached to a faucet, but it was found that a more uniform current of air can be obtained by interposing a large carboy between the apparatus and the suction pump. This makes it possible to attach also several batteries of flasks and absorption tubes to the filter pump at this point, in case a large number of samples are to be determined at the same time. The amount of air passing through the system can be regulated by opening or closing the screw clamp at Y.

Flask X contains a Bunsen valve to prevent the backward rush of air, in case something should go wrong with the suction pump. Care must be taken to prevent this sucking back, and if for any reason it is desired to disconnect a flask in the system, the aspiration is slowed down and a few screw clamps placed on the rubber connections just back of the point of detachment. The air entering the system is freed from ammonia by drawing it through a 10 per cent. acid solution.

Less trouble from frothing occurs when a flask is used which increases in diameter just above the surface of the liquid, and when the oil is blown through a glass tube with the end so constricted that the oil can be spread on the sides of the flask and over the surface of the liquid. Excessive frothing may be prevented by the addition of a drop of capryl alcohol.

After aspiration has continued during the required period of time, the system is disconnected, starting with the flask and tube farthest removed from the suction pump and working forward. It is best to have a small current of air passing through the apparatus while this is being done. The standard acid in the absorption tubes is now titrated and the nitrogen present is calculated as ammonia. To the ammonia-free extract in each flask is now added a sufficient amount of sodium hydroxide to make this approximately one-eighth normal (in these tests, 5 cc. of 6 per cent. sodium hydroxide was added), and 2 to 2.5 grams of Devarda's alloy which has been previously ground fine enough to pass a 100 mesh sieve. The flasks are immediately connected, as before described, with a new series of absorption tubes containing the standard acid, and aspiration is again continued for a period of from twelve to fourteen hours. The standard acid in the tubes is titrated against standard sodium hydroxide and the nitrates in the sample are calculated. Whenever a 100 cc. sample of the plant extract is

used, it is found best to add the sodium salts in the solid state, in order to keep down the volume. In such cases, 10 grams of sodium carbonate and 6 grams of sodium chloride per hundred cc. of plant extract is used.

In testing out the method, duplicate samples of solutions containing nitrogen, or of plant extracts, were sometimes employed in double batteries of flasks and tubes, the sodium salts being added to the samples in both series, but the sodium hydroxide and the Devarda's alloy were added to the samples of only one of the two series, and both were thoroughly aspirated. The difference in the titration values of the standard acid in corresponding tubes of the duplicate series here represented the nitrates in the samples.

The standard deviations and probable errors were calculated, in all cases, according to the following formulas:

$$\text{Standard deviation } (\sigma) = \sqrt{\frac{\Sigma(F \cdot D^2)}{N}}$$

$$\text{Probable error } (E_m.) = \frac{\pm 0.6745 \cdot \sigma}{\sqrt{N}}$$

Experimental

In testing out experimentally the accuracy of the method here described, the first step was to determine whether nitrate nitrogen could be recovered from an aqueous solution of a pure potassium nitrate. These tests were carried out in accordance with the method described. In one series fifteen samples were used, each consisting of 50 cc. of the nitrate solution. Each sample contained five mg. of nitrogen. The second series was like the first except that each 50 cc. sample contained 10 mg. of nitrogen. The results are recorded in table I.

It will be observed from table I that the amount of nitrogen recovered from each sample corresponds very well with that of the original sample. The results of all the determinations are well within experimental error with the exception of no. 6, which is approxiamtely 0.2 mg. too high. This is equivalent to only 0.28 cc. of twentieth normal acid.

The method was then tested to determine whether both ammonium nitrogen and nitrate nitrogen could be accurately determined in a mixed solution of inorganic salts. The following salts were used:

Ferrous ammonium sulphate	2.800	grams
Potassium nitrate	2.886	"
Magnesium sulphate	1.167	"
Potassium phosphate (monobasic)	0.6047	"

The salts were dissolved separately, the solutions mixed and then made up to two liters. Eight 50 cc. aliquots were taken for analysis. Each sam-

TABLE I

RECOVERY OF NITROGEN FROM A POTASSIUM NITRATE SOLUTION

SERIES I			SERIES II		
SAMPLE NUMBER	NITROGEN IN SAMPLE	NITROGEN RECOVERED	SAMPLE NUMBER	NITROGEN IN SAMPLE	NITROGEN RECOVERED
1	5.00 mg.	4.99 mg.	16	10.0 mg.	9.94 mg.
2	"	4.97	17	"	9.95
3	"	5.00	18	"	9.95
4	"	4.99	19	"	9.96
5	"	4.97	20	"	9.97
6	"	5.20	21	"	10.00
7	"	5.10	22	"	10.02
8	"	5.04	23	"	9.99
9	"	5.04	24	"	9.97
10	"	5.02	25	"	9.99
11	"	5.04			
12	"	5.00			
13	"	4.97			
14	"	4.94			
15	"	5.01			
Mean = 5.02 ± 0.01 mg. σ = 0.06 mg.			Mean = 9.97 ± 0.005 mg. σ = 0.024 mg.		

TABLE II

RECOVERY OF AMMONIA AND NITRATE NITROGEN FROM A MIXED SALT SOLUTION

NITROGEN AS AMMONIA IN SAMPLE	NITROGEN RECOVERED	NITROGEN AS NITRATES IN SAMPLE	NITROGEN RECOVERED	TOTAL NITROGEN IN SAMPLE	TOTAL NITROGEN RECOVERED
mg.	mg.	mg.	mg.	mg.	mg.
5.00	5.04	10.00	10.19	15.00	15.23
"	5.02	"	10.27	"	15.31
"	5.05	"	10.12	"	15.15
"	5.04	"	10.27	"	15.31
"	5.04	"	10.27	"	15.30
"	5.02	"	10.22	"	15.25
"	5.01	"	10.18	"	15.22
"	5.00	"	10.21	"	15.15
Mean = 5.02 ± 0.004 mg. σ = 0.018 mg.		Mean = 10.21 ± 0.012 mg. σ = 0.05 mg.		Mean = 15.21 ± 0.014 mg. σ = 0.06 mg.	

ple, therefore, contained 5 mg. of nitrogen as ammonia and 10 mg. as nitrate, by calculation. The results of the determinations are given in table II. The ammonia recoveries are in very close agreement with the calculated values but the nitrate recoveries are somewhat high. This mixed salt solution was checked for nitrates by other methods, and the results obtained were in quite close agreement with those in table II, indicating that nitrogen as nitrate other than that in the potassium salt was present in the mixed salt solution, which was not accounted for in the theoretical calculation.

Logically the next step in testing out the method was to make quantitative determinations of nitrate nitrogen in the presence of organic matter. An extract was made from 100 grams of Paper White narcissus bulbs, containing no nitrate nitrogen (as shown by the diphenylamine test on an aliquot of the tissue dried at 80° C. and ground to an impalpable dust). The extract was prepared by grinding the tissue and extracting with two liters of water. This extract was then reduced in volume by evaporation to 500 cc., and ten 50 cc. aliquots were taken for analysis. Ammonia nitrogen was determined on the first four samples, then 20 mg. of nitrogen in the form of potassium nitrate was added to each of the ten samples. The system was aspirated for 14 hours and nitrate recoveries were determined. Since ammonia as well as nitrate nitrogen is recovered by this method, it was necessary in the case of the last 6 samples to subtract the previously determined ammonia nitrogen from the total recovery in order to obtain the nitrate nitrogen added to the sample. The nitrate nitrogen and the ammonia nitrogen recovered from the extract are given in table III.

These analyses show that nitrates can be recovered quantitatively in the presence of a plant extract. It is also evident from the mean recovery (20.05 ± 0.015 mg.) and the standard deviation (0.072 mg.) that no nitrogen was obtained from the organic fractions, even though each sample contained approximately 40 mg. of nitrate-free soluble nitrogen, according to the analyses of the organic fractions of the extracts of the same plant material, presented in table IV through the courtesy of Dr. G. T. NIGHTINGALE and Dr. W. R. ROBBINS. This table appears also in Bulletin no. 472 of the New Jersey Agricultural Experiment Station.

The results of the analyses as given in table III thus clearly indicate that any nitrates present in the plant extract may be completely reduced to ammonia in an eighth normal alkaline solution, by the method here employed, without any danger of the liberation of nitrogen from the organic fractions.

To determine still further the action, if any, of a cold eighth normal alkaline solution on the organic fractions in a plant extract during the process of the reduction of nitrates to ammonia, the following experiment was

TABLE III

RECOVERY OF NITROGEN FROM POTASSIUM NITRATE WHEN ADDED TO A NITRATE-FREE EXTRACT OF PAPER WHITE NARCISSUS BULBS

SAMPLE NUMBER	NITROGEN AS AMMONIA IN THE EXTRACT	SAMPLE NUMBER	NITROGEN AS NITRATES ADDED TO SAMPLE	NITROGEN RECOVERED AS NITRATES
	mg.		mg.	mg.
1	0.44	1	20.00	20.05
2	0.41	2	"	19.97
3	0.42	3	"	20.01
4	0.41	4	"	19.97
		5	"	19.97
		6	"	20.09
		7	"	20.18
		8	"	20.16
		9	"	20.07
		10	"	20.07
	Mean = 0.42 ± 0.004 mg. σ = 0.012 mg.		Mean = 20.05 ± 0.015 mg. σ = 0.072 mg.	

TABLE IV

NITROGEN FRACTIONS OF PAPER WHITE NARCISSUS BULBS AS PERCENTAGE OF DRY WEIGHT

FRACTION	CENTERS	STORAGE TISSUE*
Total nitrate-free nitrogen	2.38	1.59
Protein nitrogen	1.59	1.25
Nitrate-free soluble nitrogen	0.79	0.36
Proteose nitrogen	0.22	0.15
Basic nitrogen	0.23	0.05
Amide nitrogen	0.13	0.07
Amino nitrogen	0.086	0.04
Ammonia nitrogen	0.008	0.003
Humin nitrogen	0.006	0.004
Other nitrogen	0.110	0.041

* Outside portions of bulbs consist of storage tissue, pure white in color. Centers are composed of immature cells and the tissue is yellow. Both regions are well defined and separated by a sharp line of demarcation.

carried out. Two series of oat plants were grown in culture solutions and were harvested just before coming into head. The plants of series I were

grown in a solution having a relatively high ammonium and a relatively low nitrate content. Ninety grams of green tissue were extracted in the usual manner. Series II was grown in a culture solution with a relatively high nitrate and low ammonium content. Eighty-three grams of the green plant tissue were extracted. Fifty cc. samples of these extracts were used in the analyses. To all samples were then added the sodium chloride and sodium carbonate, as previously described for the ammonia determination. To the even numbered flasks in the battery was added also sufficient sodium hydroxide to bring the extract to approximately an eighth normal solution. That is, the extracts in the even numbered flasks were treated as for nitrate determinations except that the Devarda's alloy was omitted. The extracts were now aspirated for a period of from 12–14 hours and the ammonia determined. The results of the tests are presented in table V, which is self-explanatory.

TABLE V

EFFECT OF EIGHTH-NORMAL SODIUM HYDROXIDE UPON THE ORGANIC FRACTIONS OF THE EXTRACT OF OAT PLANTS

	EXTRACT PLUS SODIUM CARBONATE AND SODIUM CHLORIDE AS IN AN AMMONIA DETERMINATION		EXTRACT PLUS SODIUM SALTS AND SODIUM HYDROXIDE AS IN A NITRATE DETERMINATION WITH ALLOY OMITTED	
SERIES I 90 GRAMS GREEN TISSUE EXTRACTED	SAMPLE NUMBER	NITROGEN AS AMMONIA IN SAMPLE	SAMPLE NUMBER	NITROGEN AS AMMONIA PLUS ORGANIC N DUE TO HYDROLYSIS
Oats grown in a solution with high ammonium and low nitrate content		mg.		mg.
	1	2.22	2	2.26
	3	2.24	4	2.28
	5	2.18	6	2.29
	7	2.22	8	2.29
	9	2.26		
	Mean = 2.22 ± 0.008 mg. σ = 0.027 mg.		Mean = 2.28 ± 0.04 mg. σ = 0.012 mg.	
SERIES II 83 GRAMS GREEN TISSUE EXTRACTED				
Oats grown in a solution with high nitrate and low ammonium content		mg.		mg.
	1	0.41	2	0.46
	3	0.41	4	0.42
	5	0.41	6	0.45
	7	0.39	8	0.40
	9	0.36		
	Mean = 0.40 ± 0.006 mg. σ = 0.020 mg.		Mean = 0.43 ± 0.008 mg. σ = 0.024 mg.	

From the data of table V it will be observed that the nitrogen recovered from the extracts in the even numbered flasks of series I is just slightly higher, in every case, than that recovered from the extracts in the odd numbered flasks. This indicates that the eighth normal hydroxide does have a low hydrolyzing influence upon the organic fractions of the extract; but this is so slight as to be practically negligible. The 50-cc. samples of the extracts in this series each contained, as determined by analyses, about 150 mg. of organic nitrogen. Of this amount, only 0.06 mg., liberated in the process of analysis, can be attributed to the hydrolyzing effect of the eighth normal hydroxide. This is well within the limits of experimental error.

In series II, the ammonia content of the extracts was much lower than in those of series I. In this series, as in series I, the nitrogen recovered which can be attributed to the hydrolyzing effect of the eighth normal hydroxide is practically negligible, being only 0.03 mg. per sample, although the organic nitrogen present in each sample was approximately 125 mg., as determined by analysis.

It should be pointed out that the omission of the Devarda's alloy in these tests might be a serious objection, since it is conceivable that the alloy might have some influence in liberating nitrogen from the organic fractions by increasing the catalytic action of the alkali. If this were probable, such consistent nitrate recoveries as those indicated in table III could scarcely have been obtained. However, to meet such an objection, assuming that it is a valid one, the following experiment was conducted.

Four ammonia determinations and 4 nitrate determinations were made on the nitrate-free extract obtained in the usual manner from 150 grams of Paper White narcissus bulbs. Since no nitrates were present in the extract, only the free ammonia should be recovered, on the assumption that the alkali and Devarda's alloy added to the extract in the nitrate determination can liberate no nitrogen from the organic fractions. The analyses were carried out in the manner previously described, and the data are presented in table VI.

The data of table VI again show slightly higher nitrogen recovery by the nitrate method than by the method for ammonia. The difference by the two methods, however, is only 0.05 mg. for each sample, this being equivalent to but 0.07 cc. of a twentieth normal acid. These tests, in connection with those of the preceding experiment, indicate quite clearly that the presence of the Devarda's alloy has no influence whatever in liberating nitrogen from the organic fractions of these plant extracts.

It should be noted, however, that in all the analyses of plant extracts reported here, there is always a very slightly but consistently higher nitrogen recovery when eighth normal sodium hydroxide is added to the extract

TABLE VI

EFFECT OF THE NITRATE METHOD UPON THE ORGANIC NITROGEN IN THE NITRATE-FREE EXTRACT OF PAPER WHITE NARCISSUS BULBS

EACH SAMPLE CONTAINS ONE-TENTH OF THE EXTRACT FROM 150 GRAMS OF BULBS

NITROGEN BY AMMONIA METHOD		NITROGEN BY NITRATE METHOD	
SAMPLE NUMBER	NITROGEN RECOVERED AS AMMONIA	SAMPLE NUMBER	NITROGEN RECOVERED AS AMMONIA
	mg.		mg.
1	0.73	5	0.83
2	0.71	6	0.75
3	0.69	7	0.70
4	0.70	8	0.70
Mean = 0.71 ± 0.005 mg. σ = 0.016 mg.		Mean = 0.74 ± 0.018 mg. σ = 0.017 mg.	

than when it is omitted, although the difference in the corresponding values obtained by the two methods is well within experimental error. This slight but consistent difference is undoubtedly due either to incomplete recovery of nitrogen as ammonia in the absence of the eighth normal sodium hydroxide, or to slight hydrolysis of the organic fractions in its presence. The organic fractions of some biological materials may, of course, undergo alkaline hydrolysis more readily than do those of the plant extracts here employed. It is well, in any case, to test the action of the alkali on the material in question when making nitrate determinations by the method here described, as was done in the case of the extracts from the oat plants reported in table V.

RANKER (**8**), in showing the inaccuracies of the Devarda method for determining nitrates in the presence of organic matter, found, of twenty-four nitrate-free materials tested, that glycine and urea were among those giving the highest yield of ammonia. These two compounds, as well as arginine, were therefore subjected to a nitrate analysis by the method here described. The sodium carbonate and sodium chloride solutions were added to each of the 50-cc. samples, but no ammonia was recovered from any of the materials after twelve hours of vigorous aspiration. The solutions were then made eighth normal with sodium hydroxide and one gram of alloy was added to each sample as in a nitrate determination, and aspiration was continued for 12 hours. The results obtained are given in table VII.

Approximately 1.8 per cent. of the nitrogen in arginine was liberated even by this method. This hydrolysis might be expected, since, according

TABLE VII

ACTION OF THE NITRATE METHOD ON ARGININE, GLYCINE AND UREA

	ARGININE 32 MG. NITROGEN IN EACH SAMPLE	GLYCINE 46.6 MG. NITROGEN IN EACH SAMPLE	UREA 23.3 MG. NITROGEN IN EACH SAMPLE
SAMPLE NUMBER	NITROGEN OBTAINED	NITROGEN OBTAINED	NITROGEN OBTAINED
	mg.	mg.	mg.
1	0.63	0.26	0.00
2	0.63	0.21	0.03
3	0.59	0.41	0.00
4	0.63	0.26	0.07
5	0.59	0.37	0.00
6	0.60	0.23	0.09
7	0.56	0.37	
8	0.56	0.26	
	Mean = 0.60 ± 0.007 mg. σ = 0.028 mg.	Mean = 0.31 ± 0.014 mg. σ = 0.061 mg.	Mean = 0.03 ± 0.010 mg. σ = 0.036 mg.

to PLIMMER (**6**), arginine yields ammonia by alkaline hydrolysis more readily perhaps than any other amino acid. The quantitative determination of arginine is based upon the fact that one-half of its nitrogen is liberated by boiling with alkali by the VAN SLYKE method (**10**).

Of the 46.6 mg. of nitrogen in each sample of the glycine solution, 0.3 mg., or 0.66 per cent., was broken down to ammonia. This is a much higher percentage than was obtained from any of the plant extracts studied and is an indication that with some biological materials it may be necessary to determine the ammonia liberated by the eighth normal alkali during the process of nitrate reduction and to deduct this from the total ammonia liberated during the process, as suggested above. As the last column of table VII indicates, only a trace of nitrogen was obtained from the 23.3 mg. contained in each sample of the urea solution.

From a consideration of the data presented in the tables of the foregoing pages, and of those obtained from many other tests of the method here described but not included in these tables, it is apparent that nitrates may be determined in plant tissues by this method with a high degree of accuracy, and it is hoped that with modifications the method may be generally useful in determining the inorganic nitrogen fractions of plant biological materials.

LABORATORY OF PLANT PHYSIOLOGY,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION.

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BRIEF PAPERS

EFFECT OF SUNSHINE AND SHAPE OF FRUIT ON THE RATE OF RIPENING OF TOMATO FRUITS*

The accompanying table (table I) shows high positive correlation between the number of sunshine hours and the number of days required for tomato fruits to reach full maturity after they had started to redden. This is surprising in view of the fact that light is not considered essential for the ripening processes of the tomato fruit. (1) The fruits are in fact ripened, by many commission merchants, in darkened rooms with or without the addition of ethylene. (2) DUGGAR[1] found that heat and oxygen, but not light, were factors influencing the rate of color formation.

TABLE I

COEFFICIENT OF CORRELATION BETWEEN NUMBER OF SUNSHINE HOURS AND THE NUMBER OF DAYS REQUIRED FOR TOMATOES TO REACH FULL MATURITY AFTER THEY HAD STARTED TO REDDEN

VARIETY	NUMBER OF FRUITS INVOLVED	COEFFICIENT OF CORRELATION
Baltimore	75	0.657 ± 0.044
Marglobe	73	0.400 ± 0.066
Red Pear	255	0.555 ± 0.029
Ponderosa	42	0.680 ± 0.053

The results reported herewith were obtained in the Purdue greenhouses during the fall of 1927 and winter of 1928. Each plant was grown in a galvanized iron pot containing 40 pounds of rich loam soil. The plants were not allowed to become starved for any of the mineral elements nor to suffer from lack of moisture. The night temperature was regulated at approximately 60° F. and the day temperature at 70–75° F. The higher day temperature was maintained only on sunny days. Though the high correlations secured seem to indicate that a greater amount of sunshine may have been responsible, under the conditions of this experiment, for the more rapid ripening of the tomato fruits it is nevertheless possible that the higher temperatures may have been entirely responsible. It is, however,

* Published with the approval of the Dean of the School of Agriculture and the Director of the Agricultural Experiment Station of Purdue University.

[1] DUGGAR, B. M. Lycopersicin, the red pigment of the tomato, and the effects of conditions upon its development. Washington Univ. Studies **1**: 22–45. 1913.

TABLE II

COEFFICIENT OF CORRELATION BETWEEN SHAPE AND NUMBER OF DAYS REQUIRED FROM POLLINATION TO MATURITY FOR TOMATO FRUITS

VARIETY	NUMBER OF FRUITS USED	COEFFICIENT OF CORRELATION	MEAN SHAPE OF FRUIT	NUMBER OF DAYS FROM TURNING TO RIPENESS	NUMBER OF DAYS FROM POLLINATION TO MATURITY	MEAN WEIGHT (GRAMS)
Baltimore	75	-0.130 ± 0.077	1.41 ± 0.002	4.93 ± 0.12	66.3 ± 0.70	142.3 ± 4.2
Marglobe	73	0.002 ± 0.079	1.32 ± 0.009	5.42 ± 0.13	64.6 ± 0.52	153.8 ± 3.96
Red Pear	255	0.149 ± 0.041	0.76 ± 0.003	5.88 ± 0.09	56.0 ± 0.17	11.8 ± 0.11
Ponderosa	42	-0.224 ± 0.098	1.50 ± 0.013	7.00 ± 0.21	63.7 ± 0.56	226.2 ± 6.96

plausible that the greater amount of light favored the development of the red color of the tomatoes in this experiment as they were ripened on vines which were grown during the fall and winter under inadequate light conditions. DUGGAR did not test the effect of light on the rate of ripening of fruits that were still attached to the vines and although most of the fruits were grown in the greenhouse he does not state that light may have been a limiting factor for growth. Moreover the temperatures, 60–75° F., maintained during the Purdue tests were considered by DUGGAR as satisfactory for the formation of the red color of tomatoes.

There was no significant correlation between the shape

$$(\text{shape} = \frac{\text{equatorial diameter})}{\text{polar diameter})}$$

of fruit and the rate of ripening as is shown in table II, although the tendency is for the rapid ripening of ovate fruits (Red Pear) and for the slower ripening of oblate fruits such as Baltimore and Ponderosa.

There seems to be some correlation between the weight of fruit and the number of days required for maturity although the Baltimore is a notable exception and the other data are not altogether significant.

The ovate fruit shape also seems to be correlated with the smaller fruits (*i.e.*, those that weigh less) although the Marglobe fruits prove an exception to this rule. This agrees with the results secured by LINDSTROM[2] in working with hybrids of ovate and oblate varieties of fruit.—C. L. BAKER and H. D. BROWN, *Purdue University.*

TWO PLANT MATERIAL DRIERS

(WITH FOUR FIGURES)

In the preparation of plant material for the study of enzyme content it sometimes becomes necessary to hasten the process of drying if one wishes to retain the enzymes as they are in the living plant. The drawings on another page show a drier adapted to the use of heat, and another to the use of sulphuric acid. In both, the "dead" air around the material is obviated, in one case by the use of a fan, and in the other by the movement of the material in a rotating basket.

In the first (fig. 1), warm air is forced directly over the material, and no "dead" air is allowed to retard the drying. Most plant leaves dry within four hours at a temperature of 42° C. Quiet unheated air requires about 24 to 36 hours for a less satisfactory drying. The walls are of beaver board one half inch apart. The cross partitions are of copper wire fly-screen. A longitudinal partition separates them at the middle, making six compartments in all.

[2] LINDSTROM, E. W. The inheritance of ovate and related shapes of tomato fruits. Jour. Agr. Res. 34: 961–985. 1927.

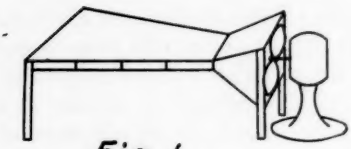

Fig. 1.

Fig. 3.

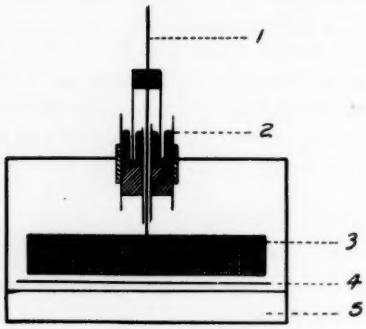

Fig. 2.

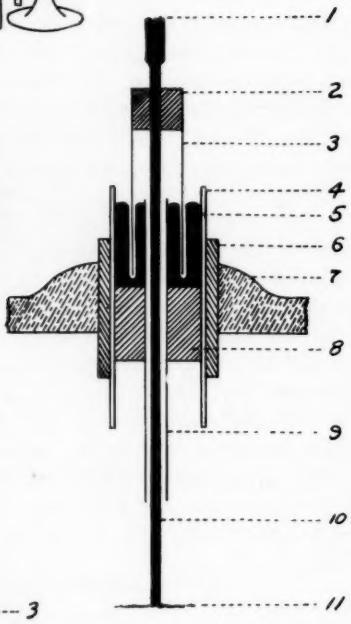

Fig. 4.

FIG. 1. Hot air plant drier.

FIG. 2. Section of sulphuric acid plant drier. 1. Iron shaft. 2. Mercury seal. 3. Rotating basket. 4. Hardware cloth screen. 5. Sulphuric acid.

FIG. 3. Detail of rotating basket. The top circular piece is not shown.

FIG. 4. Detail of mercury seal. 1. Attachment of shaft to motor. 2. Cork stopper through which the shaft is tightly fitted. 3. Glass tube rotating with the shaft. 4. Larger glass tube. 5. Mercury. 6. Cork with hole to accommodate glass tube "4." 7. Lid of desiccator. 8. Cork. 9. Glass tube. It should extend above mercury. 10. Shaft. 11. Attachment of shaft to basket.

The heating element is made of three feet of coiled wire similar to that used in hot plates. It is wound around a transite piece 2 x 8 inches. The fan is 9 inches in diameter, and rotates within the hood.

The "cold" drier (fig. 2) is not so rapid but may be employed at or much below room temperature. Its efficiency lies in the moving basket. We have found the electric motor stirrer most suitable for power. It is attached directly to the shaft and rotates at a speed of 70 r. p. m. The basket (fig. 3), has four compartments, and is made of one-half inch mesh hardware cloth. Two circular pieces held two inches apart by a cross of the same material are closed in by an outside band. If the cross-pieces are cut so as to allow the wires to extend on each side, and these used to bind them to the top and bottom of the basket, a solid construction is assured. A portion of the top is cut out above each compartment, a shaft soldered in the center and it is ready for service. A cloth lining may be needed when some material of smaller pieces is being dried.

Fig. 4 shows a detail of the mercury seal. If warm paraffin is poured into the mercury moat and out again it will be made mercury-tight.

The time of drying is reduced about two-thirds over that of non-moving materials above sulphuric acid.

Experiments are under way to test the relative merits of these two driers for drying plant materials for use in the study of plant enzymes.—A. G. Wood, *Grove City College, Grove City, Pa.*

NOTES

Summer meeting of the American Society of Plant Physiologists.—At the invitation of the Purdue Section, the summer meeting of the Society was held at Purdue University, Lafayette, Indiana, September 4 and 5. About fifty were in attendance, mostly from the states of Ohio, Michigan, Illinois, Indiana, and Wisconsin.

On September 4 a program of short papers was given by members of the Purdue staff, explaining investigations and field plot work. Mr. J. F. TROST discussed the response of corn hybrids to fertilizers. Mr. L. P. MILLER gave the results of his studies on the effect of manganese deficiency in sand culture. Dr. E. B. MAINS reviewed the results of studies concerning physiologic specialization in the rusts. Mr. D. K. DOAK gave the results of his investigations concerning the effect of mineral nutrition on the rust reaction of wheat. Dr. J. H. MACGILLIVRAY summarized the results of his studies concerning tomato quality. Prof. L. P. CULLINAN and Mr. J. L. SULLIVAN gave the results of their studies concerning the nutrition of apple trees. Prof. H. P. BREWER described methods for the purification of the virus of tomato mosaic. Mr. L. M. BUSHNELL described the dominant soil types in the vicinity of Lafayette. Prof. S. D. CONNER outlined the agronomy field plot experiments. Following the dinner, which was held at Lincoln Lodge, Dr. C. A. SHULL discussed the present status of the journal, PLANT PHYSIOLOGY, and plans for its future development.

September 5 was spent in field trips. In the morning the Soils and Crops experimental farm east of Lafayette, and the Animal Husbandry farm north of West Lafayette were visited, and breeding and fertility experiments with corn were studied. Following a lunch at the Fowler Hotel, Prof. E. J. KRAUS, President of the Society, discussed the relationship of plant physiology to applied botany. In the afternoon the orchards of the Horticultural Department west of Lafayette were visited, and pruning and fertility experiments were studied. Taken as a whole, the meeting was very successful, and the Purdue Section is to be congratulated on the manner in which the arrangements were carried through.

The Fifth Annual Meeting.—The annual meeting to be held at New York in December, 1928, is the fifth annual meeting of the Society. As this note is being prepared, the program arrangements are reaching completion. Members who can attend these meetings should offer their best contributions to the Program Committee. The main outlines of the program indicate a fine meeting, with two excellent symposia, one on Cell

Physiology, and the other on Radiant Energy Relations of Plants. These are held jointly with the Physiological Section of the Botanical Society of America.

The headquarters hotel has been announced as the Lincoln Hotel. Every member who can do so should attend the New York meeting. It is one of the quadrennial meetings of the American Association for the Advancement of Science, and offers an opportunity for scientists of all fields of work to consolidate their forces for the promotion of greater freedom and greater privileges for the leaders of scientific progress.

Those who attend the meeting should plan to be present at the annual dinner for all plant physiologists. The announcement of the first STEPHEN HALES prize award, and the award of the third CHARLES REID BARNES Life Membership should make this a memorable occasion.

The New Constitution.—Copies of the new constitution have been sent out to the members of the Society by the Secretary. The growing activities of the Society have made necessary a more detailed instrument than the one under which the Society has been operating. The provisions should be examined critically by all members, to uncover any defects which may still be present. The instrument will be more difficult to change after adoption than before, and every effort should be made to bring it to perfection before the vote is taken.

Size of Manuscripts.—The matter of size of manuscripts suitable for publication in PLANT PHYSIOLOGY has been mentioned by several members. The value of a manuscript is obviously not determined by its length, and the editorial committee has not set any definite limit upon the size of papers that may be published. Some short papers are much more valuable than other longer ones, and the editors are far more concerned about the quality than about the size of papers submitted for examination. However, there are limitations to the volume of material which can be printed. PLANT PHYSIOLOGY can use enough manuscript each year to print about 500 pages per volume without deficit at its present rate of income. That means that we could use 16–18 papers, each printing up thirty pages, within a year. But if the papers run 10 to 15 pages, we can use 40 to 50 in a year. Many a long paper can be improved by condensation, and elimination of material with which everybody is familiar. There is often a tendency to restate the obvious, and to spin out introductions to unnecessary length. The main need is for papers that are well organized, full of new information, thoroughly digested, and clearly written. The editorial board will probably continue to accept papers of almost any length that measure up to their

standard of value. But contributors are asked to keep in mind the limitation of size on the annual volume of publication, and to make papers as concise as good presentation permits.

Carbon Copies of Manuscripts.—It is probable that most of the authors who submit manuscripts to PLANT PHYSIOLOGY retain carbon copies of their papers. This is a desirable practice. So far, there has been no loss of a manuscript in the mails, but in passing around the editorial board each paper makes a journey of six thousand miles. Each paper is mailed five times, and there is always some risk of loss. The seriousness of such a loss will be minimized if the author has a carbon copy in reserve. It also facilitates correspondence between editor and author about changes in the paper, if they seem desirable, and does away with mailing of manuscripts with proofs.

Costs of Publication.—In order that the members of the American Society of Plant Physiologists may be informed as to the costs of publication of the official journal, the following figures are presented, showing the various items which make up the cost of a single issue of PLANT PHYSIOLOGY. The April number is used as an example:

10 pt. text	$372.78
8 pt. text	48.84
Tabular	99.24
Blanks	8.70
Engraving	105.74
Changes	22.50
Stencils	.48
Postage	11.13
Cover	11.17
Wrapper and mailing	3.50
Reprints	8.30
Total	692.38

The two items which need most careful attention are those for tabulated data, and for the illustrations.

Cost of Reprints.—The high cost of reprints is an evil which we have not been able to abate. Authors have a right to know the basis upon which charges for this service are calculated, so that they can estimate approximately the cost of the reprints ordered. We are presenting, therefore, the rates now charged by the Science Press Printing Company for the printing of separates for authors.

	1 page	4 pages	8 pages	16 pages	32 pages
50 copies	$3.00	$3.70	$5.40	$7.60	$15.20
100 copies	3.25	4.10	6.00	8.50	17.00
Additional 100	.50	.75	1.20	1.80	3.60

Covers, 50 for $2.75; additional covers at the rate of $2.00 per hundred. Self-covered reprints cost $1.50 in addition.

A 2-page reprint is charged at the 4-page rate, a 6-page as 8, 10- and 12-page as 16 pages. A 20-page reprint is counted as a 16-page and 4-page combined. Authors who order reprints at these rates receive in addition to those ordered, 25 copies without covers, gratis. In case the author does not place an order, no reprints are made up of that particular paper.

Portrait of Timiriazeff.—The portrait of TIMIRIAZEFF in the April number is only the first of a series of portraits of famous plant physiologists to be published in PLANT PHYSIOLOGY. Believing that there are a number of plant physiologists who would like to place these portraits in frames and hang them upon the walls of their class rooms and laboratories, a few copies were made without the plate number and journal name. While they last, copies may be obtained from the editor of PLANT PHYSIOLOGY for twelve cents. As the number printed was not large, those who desire copies should send for them early.

Joseph Tooker Rosa.—It is with much regret that we have to record the death of Dr. JOSEPH T. ROSA, one of the staunch supporters of the Society, who died suddenly at his home in Davis, California, on August 8, 1928. Although but 33 years old, he had contributed many valuable papers on the management of truck plants, such as potatoes, sweet potatoes, tomatoes, melons, and spinach. A South Carolinian by birth, he was reared in a region where truck cropping was an important industry. His early contacts with the problems of production and storage of vegetable crops no doubt gave him the incentive to his scientific career. He received his first degree from Clemson at the age of 20, and his M.S. two years later from Iowa State College, while occupying a position as instructor in crop plants. During the succeeding five years he was a member of the horticultural staff at the University of Missouri, where he received his Ph.D. in 1922. During the last six years he has been associate professor of truck crops at the University of California, and associate plant breeder in the California Agricultural Experiment Station, at Davis, California. He will be greatly missed by his many friends.

Harris Miller Benedict.—The tragic death of Dr. H. M. BENEDICT, Professor of Botany at the University of Cincinnati, in an automobile col-

lision on October 17, while driving from his home to the University, came as a shock to all of his friends. Dr. BENEDICT has been a member of the American Society of Plant Physiologists for several years, and was much interested in the growth and development of the organization. Recently he had been appointed as one of the representatives of the Society on the Council of the American Association for the Advancement of Science. He was also a generous contributor to the STEPHEN HALES prize fund.

Professor BENEDICT was born in Illinois, received his collegiate training at Doane College, and at the University of Nebraska, taking his M.S. degree at the latter institution in 1897. A few years later he became a member of the faculty of the University of Cincinnati, and has been the leader of the development of the botany department there for over a quarter of a century. The department grew strong under his leadership, and the students and faculty members who worked with him caught the inspiring spirit of his life and work. They loved him for his fine sympathy and understanding, and for the rare quality of his friendship. And they loved botany, for he presented it as a great humanistic science.

While he received his Ph.D. degree from Cornell in 1914 in plant physiology, he was interested in a much wider field. He loved birds, knew them intimately, and was the originator and director of the Emery Bird Reserve, the first municipal reserve of this nature to be established. He was also interested in botanical gardens, particularly in an Ohio botanical garden, and as director of the Ohio Botanical Garden Society, was working for the establishment of research botanical gardens to solve the plant problems of industry.

A man of fine spirit, courteous, kind, sympathetic, enthusiastic, it will be difficult to replace him; his loss will be felt keenly among American botanists.

International Critical Tables.—The fourth volume of International Critical Tables has come from the press of the McGraw-Hill Co. It contains phase equilibrium data, including those for triple points, transition points, and melting points at ordinary and low temperatures; the effects of pressure upon the melting and transition points; volume changes on melting and transition; directly measured compressibility and thermal expansion differences. There is also a section dealing with the properties of silica, and a valuable freezing point-solubility table. The following sections present data on the solubility of salts, strong acids, and bases in water; and the solubility of organic substances and weak electrolytes in water. Data on the freezing point lowering of aqueous solutions of inorganic strong electrolytes and all salts are given. These data occupy the

first 429 pages of the volume. A brief section considers the data on osmotic pressure of cane sugar solutions, colloids, etc.

The later pages of the volume are devoted to the properties of surfaces, (surface tension, surface energy and related problems), tensile strength and angle of contact, methods of measuring surface tension, and tables for the interfacial tensions of solid/liquid and liquid/liquid interfaces. An important section by T. Fraser Young and William D. Harkins presents surface tension data for certain pure liquids between 0° and 360° C., and for all types of solutions at all temperatures.

A temporary index to volumes I–IV inclusive has been prepared, and a sheet of errata for the first 3 volumes comes along with volume IV. The set lacks only one volume of completion up to the end of 1923. Annual volumes beginning with 1924 will keep the information up to date. Every research laboratory needs this excellent reference work.

Handbook of Chemistry and Physics.—The thirteenth edition of this handbook, published by the Chemical Rubber Publishing Co., Cleveland, Ohio, has just appeared. It contains about 100 pages of new material, and various improvements in the tables. The regular price is $5.00, but students are permitted to obtain it at half price. This handbook has been a valuable aid to students during the last 15 years, and deserves its popularity.

A Textbook of General Botany.—The Wisconsin textbook of general botany appears in a revised edition, with numerous small changes, but without radical departure from the presentation in the previous edition. It has many features to commend it, and will probably continue to be used rather widely. It is a Macmillan publication, and is listed at $3.75.

A Laboratory Manual of Botany.—This laboratory outline of general botany by Emma L. Fisk and Ruth M. Addoms, is designed to accompany the Wisconsin textbook of botany. There are 36 exercises, 13 of which are devoted to a general study of the seed plants as organisms, and 23 to a survey of the plant kingdom. At the close of the outline there are some helpful suggestions for the selection and preparation of materials used in the laboratory. This manual will be welcomed, not only by those who use the general textbook of botany which it parallels, but by those many teachers who want a workable outline for the laboratory work in botany. It is reasonably priced, $1.00, and is a Macmillan publication.

Elements of Botany.—To meet the need for a text covering an introduction to botany in a single semester, R. M. Holman and W. W. Robbins

have abridged their textbook of general botany. The general arrangement is much the same as in the larger volume, and also much like the Wisconsin text in approach and arrangement of chapters. The treatment is brief, but 15 chapters, which presents the essentials of a beginning course in an attractive manner. The book is published by Wiley and Sons, at $2.75, and is well adapted to the one semester introduction.

Statistical Methods for Research Workers.—A second edition of R. A. FISHER's monograph on statistical methods has appeared from the press of Oliver and Boyd, Edinburgh and London. Professor FISHER is chief statistician of the Rothamsted Experimental Station, and his experience in handling small sample problems is placed at the service of those who need statistics in the interpretation of their results. Plant physiologists often find it advantageous or even necessary to use statistical methods in analyzing the variable results of experiments. FISHER's work is a very valuable aid in the analysis of variability, in testing the significance of means, and in determining coefficients of correlation. The 6 tables included in the text are reproduced at the end of the book in folding copies, which may be detached and mounted for convenient consultation. The price of the book is 15 shillings net. Orders should be sent to Oliver and Boyd, Tweeddale Court, Edinburgh.

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